Personal Science Guide to the Microbiome

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Table of contents

1 Intro

> **!** Important
>
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Science is the systematic and open-minded pursuit of truth through a never-ending process of systematic experimentation. It's open to everyone and can be applied to any situation, including situations that affect you right now. It's *Personal Science* when you use the scientific method to discover important insights about the wellness and performance of yourself and those around you.

This book will introduce you to one exciting area of personal science: understanding your own microbiome. It's based on my own experiences collecting and studying hundreds of my own samples, and thousands from other people. Learn everything I did, and how you can do it yourself.

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Part I

General Overview

2 Getting Started

The term "personal science" was first popularized by the late Seth Roberts, an Emeritus Professor in the Psychology at University of California, Berkeley. His best-selling book[1] and popular blog[2] insisted that much of modern science is too complicated for its own good, that interesting and practical results are often best achieved through personal experimentation. Through multiple examples from his own self-experiments, he used his own data to show non-obvious treatments for better sleep (skip breakfast), lower depression (faces in the morning), and many other situations.[3]

Most of the examples in this book are based on over 600 near-daily samples I took of my own microbiome over a three year period. Inspired by an experiment conducted at MIT[4], during most of that time I also carefully tracked the food I ate, my sleep, and other variables like activity and 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 traveling to a new place. 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.

This book tells you what I learned – and how you can learn too.

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[1](S. D. Roberts 2007)

[2]His blog, active until his death in 2014, is actively discussed on a Facebook Community: https://sethroberts.net/2016/01/13/seth-roberts-community-on-facebook/

[3]S. Roberts (2004)

[4](David, Materna, et al. 2014)

3 What is Personal Science?

Although the techniques of science are useful in all aspects of life, many people are attracted to Personal Science out of concern over a personal health issue.

Most of us grow up believing in *experts.* Whether it's a proclamation from the government, a highly-regarded book, a credentialed doctor, or an experienced family member, it only makes sense to rely on others who have spent more time with the situation than you have, or who have gained a reputation for reliably solving similar problems.

But what happens when experts disagree? Of course, you can simply choose to believe one or another based on some reasonable criteria, like their track record with treating problems like yours.

Unfortunately, many people find themselves suffering from a chronic condition for which expert advice seems to fail. One doctor says "do this" and another says the exact opposite. One treatment seems to work for while and then it no longer does. Sometimes the symptoms seem to disappear at random, despite undergoing no treatment at all. Five doctors give six different suggestions.

3.1 How to Begin

If you suffer from a chronic condition, one of your first struggles is simply *how bad is it?* What is the precise version or name of this disease? What makes you different from a healthy person, or from the healthy person you used to be? Are there other people with the same condition, and if so, how does your situation compare to theirs? Are you getting better or worse?

In other words, you want to know the *context.* The first step in any treatment plan requires that you understand how you compare…to healthy people, to those who have the same condition as you do, to people who have partially or fully recovered. Are you improving or deteriorating?

Even symptom tracking is just one aspect of the question of context. I want to know more precisely the conditions under which my problem gets better or worse. In other words, what is the *context*? (e.g. are my migraines triggered by high altitudes, by caffeine, by stress, by something else? All of these are just other ways of saying "context").

One simple example: what's the best way to treat a headache? There's no good answer to that question unless you understand something about the context surrounding the person involved. The appropriate response will depend on whether he or she:

- gets headaches all the time.
- rarely gets headaches.
- drank heavily the night before.
- recently ate raw seafood from a street vendor.
- Underwent a course of antibiotics for a tick bite last summer and seemed to get better until now.

We know intuitively that each medical situation depends on the circumstances. Doctors are helpful partly because they've seen so many other cases that they can quickly focus attention on the aspects that are important to a specific individual. In other words, doctors are trained to recognize the full *context*, to see how this situation compares to others.

3.2 Reference Values

Much of our understanding of context is driven by reference values. A doctor knows whether your cholesterol is high or low based on large population studies of other people. Every health study is essentially just a way to calculate reference values: of the n people exposed to this treatment, some fraction will improve. If that fraction is large enough, we say the treatment works. If not, the treatment doesn't work.

So the real question in any medical condition is: what is the reference value? What is the standard by which I am judging my current condition?

For many (most) situations, the reference values have been pre-computed based the medical community's long experience treating patients like you. We know that X% of people with your type of cancer respond well to this drug. We know that Y% of people who smoke develop this disease. And on and on.

But for some situations – like data from microbiome tests – there is no reference value. Nobody knows what a "healthy" microbiome looks like. We need more data before we can say definitively that such-and-such abundance levels are "healthy" or "unhealthy".

In other cases, there are reference values for the general population, but not necessarily for you. The average height of a 3-year-old girl, for example, is based on data from umpteen thousands of 3-year-olds, but what about among 3-year-olds of your ethnic group, or your family, or people of your socio-economic class, or those in your neighborhood? Whether to consider *your* 3-year-old for special treatment depends entirely on which reference group you are using.

How can we get those reference values?

In other cases, a treatment may be too new, or too crazy, for there to be reference values. A terminal cancer patient who tries an experimental treatment, for example, is living in a world of unknown reference values. Importantly, after they try the treatment, they become one of

the reference values. And that's great! we now have a reference value for that treatment — but only if somebody bothers to record it. Often that data simply falls on the floor with nobody to catch it.

3.3 Quantifying the anecdotal

If the results of a treatment are not recorded, we still have reference values. People still rely on word of mouth — anecdotes — when looking for new treatments. But those reference values are anecdotal. You regularly hear stories of the form "I tried X and it worked for me". Hear enough of those stories and you may want to try it yourself. But how many of those stories constitutes "enough" to try for yourself?

What if there were a common way for everyone who tries X to record their results quantitatively?

That's the idea behind symptom tracking, and it's a nice start. Some companies try to add fancy additional features on top, like using machine learning to try to guess better than you can alone about the various correlations found within your data. Many companies go this direction — gather enough data, either from yourself or from others, so that we can predict the causes for various states. Again, that's interesting and it's a nice start, but it's limited.

What you really want — and the key, original idea behind Personal Science — is to let you take that quantitative data and *compare* it to others: others like you, people who you consider to be just like you except for such-and-such symptom.

Now, in some cases, a symptom tracking or quantified self product will let you see yourself compared to *an aggregated* summary of all other users. Fitbit might let you compare yourself to all those of your sex or age, for example, or maybe those in your geography. This is a good start.

But what if you could choose your own subset of users with whom you want to compare yourself? Because only *you* know which type of person you identify with, or to which type of health condition you want to belong, Personal Science lets you analyze and study the data as a whole.

That's why it's *personal* – it's about the one, unique data point that is you – and why it's *science* – democratize the quantitative tools of science to let you understand your condition, for yourself.

Part II

Science

4 The Science of the Microbiome

4.1 Biology basics

With all this complexity, how do you begin to study the abundance of life around us? And then, how do we apply what we know about the zillions of organisms *around* us, to how they relate to what's *inside* us?

Like life itself, biology is a very broad field. Fortunately, despite the incredible variations, scientists have discovered a few simple traits and rules that apply to every life form. For the special life form *homo sapiens*, we have also learned a number of simplifications that will let us talk in more detail later.

4.1.1 The Basics

The study of biology starts with the cell, those tiny self-contained blocks that are the very definition of life. From the most humble microbe to the biggest animal, every living thing is made of these structures, which are really just miniature chemical reactors that pull external molecules from their environment and reassemble them in ways that perpetuate the reaction.

Everything in the universe tends over time to fall into disorganized entropy, but cells contain many tricks, honed over billions of years of evolution, to thrive. Despite the diversity of life, a surprising number of those tricks are shared by all cells: a wall to protect and allow exchanges with the outside world, a means of storing information through DNA, and of course a process of reproduction.

The biggest technical difference among cells is not size or even function, but rather the distinction between two broad categories: eukaryotes, which are the cells of everything from corn plants to humans to fungi and amoebas, and prokaryotes, which are always single-celled bacteria and other microbes. It's interesting enough that all life could be characterized into these distinct groups, but if you look at the DNA that defines each cell, you will find some other odd differences that hint at more refined relationships among living things.

A cell's DNA contains all the information needed to create another copy of itself; even the instructions for *how to do the copying* are just a sequence of predictable DNA letters written somewhere in the genetic code of all cells. This very important copying function is performed by a *ribosome*, which is a complicated but well-studied part of every living thing. Because the

ribosome has such a fundamental function, it tends not to fall prey to many mutations over time; after all, a single DNA letter change in the ribosome is almost always fatal to the entire cell. But every so often — maybe every few million years — there *is* a mutation in some part of the ribosome, and this leads us to a clever way to understand better how living things are related to one another.

Humans and monkeys, for example, may differ in many different parts of their DNA, but their ribosomes are nearly identical. In fact, the ribosomes of all mammals and even all vertebrates are virtually the same. Well, there are some differences, but interestingly the differences between large, obvious groupings like vertebrates or invertebrates are much more significant than the differences between different vertebrates, or between mammals or other creatures.

In fact we can even quantify the differences, and scientists over the years have done exactly that. The ribosomes of humans and monkeys, for example, *are* different in only 10 places — practically nothing in a molecule that consists of a few thousand nucleotides connecting dozens of proteins. Similarly, the ribosomes of vertebrates and invertebrates are different in perhaps 100 places – clearly much more significant than the differences within each grouping, but still not terribly different relative to the entire ribosome.

The ribosomes of prokaryotes and eukaryotes, on the other hand, can be quite different: perhaps 1000 places (to continue this very-rough-but-sort-of-useful metric). The point is that even at the molecular, ribosome level, we can see obvious genetic differences even if the physical differences between two organisms aren't necessarily obvious at first glance. A one-celled eurkaryote, like an amoeba or algae, for example, might seem like it should share something in common with a one-celled prokaryote, but looks are entirely deceiving: nobody looking just at the ribosome could possible mistake these as similar.

Now, scientists have mapped the differences in ribosomal structure among nearly all living things and this general rule always applies: the groupings of life forms are directly related to the similarities or differences in their ribosomes.

Meanwhile, scientists have made estimates of how long it takes, given various assumptions, for a series of step-by-step mutations to result in a differently-sized ribosome. In other words, using some basic chemistry that is easily demonstrated experimentally in a lab, we can offer some reasonable guesses for the number of generations it would take for a given level of random mutations to result in the differently-sized ribosomes we see in nature. Add it all up, and behold: you can see a reasonable fit with the clues we have in the fossil record and the geological record for the same creatures.

None of this is perfect, of course, but the point is that we have a crude way to quantify how different one organism is from another and, if you like, we can guess how long it would take for a single common ancestor to accumulate enough random mutations to account for the differences between any two life forms.

So far so good. Next let's imagine we have a circle, where a single cell begins in the middle, divides into two cells, and those cells divide, etc. for zillions of years until there are clear

ribosomal differences between each line. Let's call this a family tree and take all known life forms and spread them into this circle.

If you do that, you'll find that the number of mutations necessary to generate all the variation found in eukaryotes – everything from corn plants to people – would take up only a tiny sliver of that circle. The rest of life — in particular the microbial life of prokaryotes – is so unimaginably diverse, that a space alien looking at earth's lifeforms could well conclude that the differences between humans and corn plants aren't significant enough to worry about.

That's how complicated the world of bacteria can be.

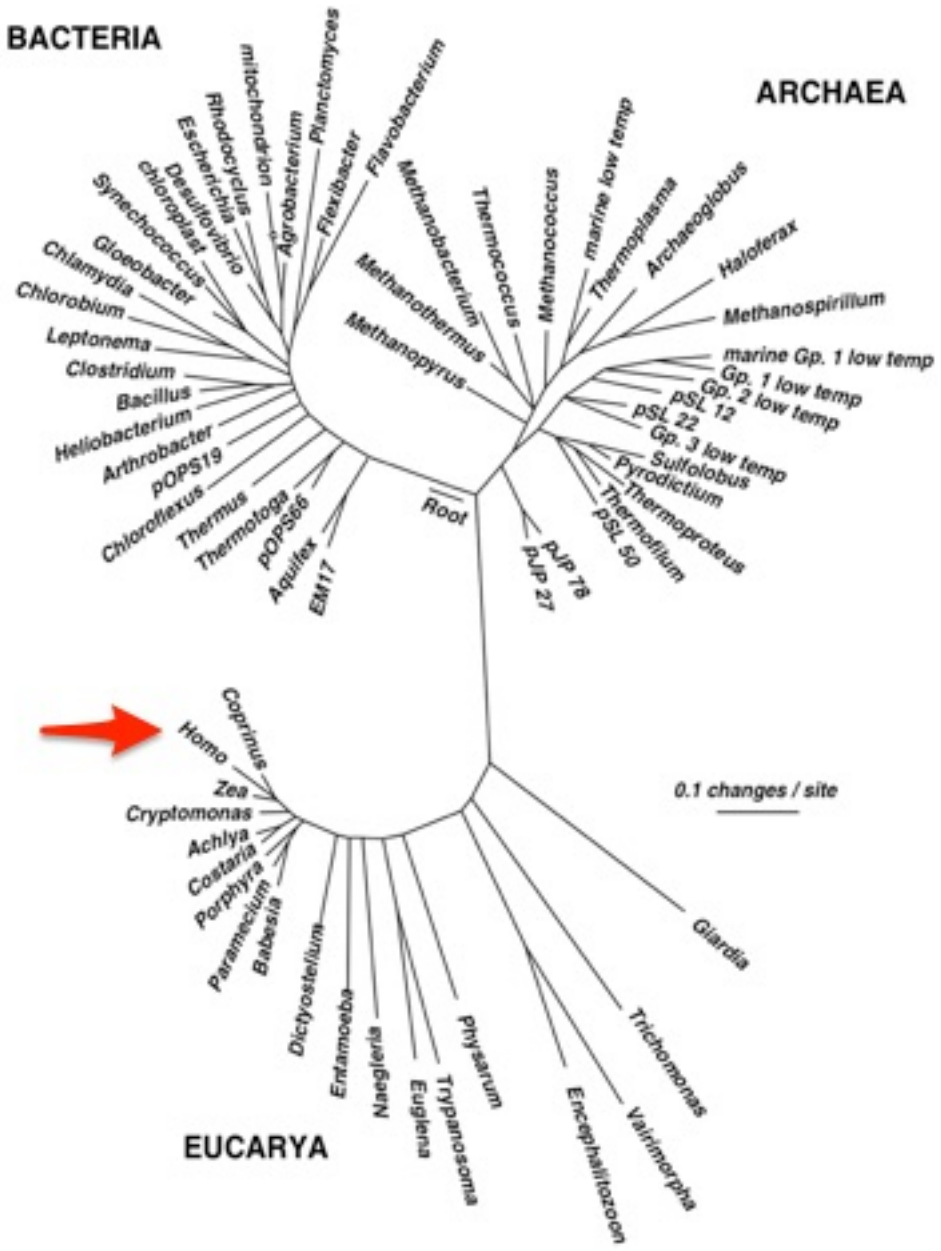


Figure 4.1: Humans are only a tiny piece of the explosive variety of life.

4.1.2 Taxonomy

How do you talk about the relationships between various different life forms?

A taxon is a simple unit of life. A *homo sapiens* is a taxon, but so is a primate. A mammal is

a taxon too. It might seem odd in the ordinary biological world to bother using the same term 'taxon' to refer to all of those units, but for bacteria and anything that reproduces asexually, it's an important distinction because often, taxonomists don't agree about whether a group of organisms is part of the same taxon or not.

Since Carl Linnaeus in the 1700's, the science of taxonomy divides all life into seven major categories: Kingdom, Phylum, Class, Order, Family, Genus, Species (which I was taught in sixth grade to remember by the mnemonic "King Philip Came Over for Girl Scouts").

Bacteria make up their own kingdom. Just as the animal kingdom includes everything from humans to jellyfish to beetles, the diversity of bacterial life is enormous, a point which can't be emphasized too much. This is true at every rank in the taxonomy. Even two organisms that are the same at a lower rank, like genus, might have radically different affects on the human body, just as a member of the animal genus Canis could be anything from a wolf or coyote to a Chihuahua.

You cannot mix and match these ranks. If you know something about the number of organisms in one genus, for example, this is meaningful only in comparison to the numbers of another genus. Keep that in mind during our analysis.

4.1.3 Microbiology

Before we get to the nuts and bolts of analyzing the microbiome, it's helpful to review a few basics of microbiology.

This section explains more about cells, but now from a chemical point of view. It's through identifying these chemicals that we are able to understand how the entire system works. Chemists and biologists have developed many brilliant techniques for identifying these processes, nearly all of which take place at microscopic levels. How we are able to tell what's happening is a subject worthy of its own book, but here we will concern ourselves with understanding how we are able to convert the happenings in the physical world of the cell, to the software world, where we can do the analysis.

A few questions to answer:

- How do we see things at such a tiny scale? How can we be confident that what we see is real?
- How does Next Generation Sequencing work?
- What is a gene, how it creates proteins, and why that matters
- what is a SNP?

Think of cells as self-contained factories that accept tiny chemical raw materials from the outside, process them, and then output byproducts. There is a whole, well-developed chemical explanation for this which we won't detail, but this long chain of inputs and outputs, carried from cell to cell, is key to the working of every living thing, including humans. The various

chemicals passed from cell to cell carry raw materials needed for life, but they also carry *information* that tells other cells what to do.

All life runs on three chemical building blocks: DNA, RNA, and proteins. Each of these is an arbitrarily-long chain of repeating molecules called *nucleotides* (DNA or RNA) and *amino acids* (proteins). Due to constraints on the way atoms interact, the set of building blocks is fixed. All DNA is composed of only four nucleotides: adenine, thymine, guanine, and cytosine, represented by the letters A, T, G, and C. RNA is composed of the same molecules, except that uracile (U) is substituted for thymine.

Similarly, proteins are constructed with only 20 different amino acids, which can again be represented by a short three-character abbreviation.

The correspondence between these different proteins and combinations of DNA or RNA is referred to as the genetic code.

As a programmer looking through all of this, you may immediately be inspired to write your own software version of this. After all, the remarkable consistency between all of these building blocks cries out for manipulation by computer.

In fact, that's exactly what bioinformaticians do, and numerous software packages have been developed to make it easy to treat these building blocks of life like ordinary computer strings.

Perhaps the biggest challenge is the volume of data to be handled, which can easily be measured in gigabytes for a simple organism, but can require entire server farms in the case of some real-world biological systems. For that reason, much of bioinformatics is about optimizations to improve the speed of processing a large data set, or to simply the presentation in a way that can reveal the most biologically interesting aspects of a problem without wading in over-complexity.

One special protein, DNA, can store information.

5 Microbes Everywhere

Living microbes are found everywhere on earth, often in surprising places. This section looks at some examples of how ubiquitous and hardy they can be, both in nature and on our bodies. We'll also discuss the technologies used to study the human microbiome.

The most important parts of our world are invisible. We can't see air, but we can't live without it. Similarly, our bodies are literally bathed in living, eating, reproducing lifeforms that we can't see but that have profound effects on all that we do.

Life is tenacious, finding its niche, fighting for it, and stubbornly holding on in every environment it encounters. Living organisms inhabit the sky, deep underground, in the most barren habitats cold or hot anywhere on earth. The vast majority of these are microbes, so small we can't see them, but small doesn't mean irrelevant. In fact, the more that science understands about the invisible microbial world, the more it becomes clear that these uncountably numerous creatures exert a much bigger effect than we think.

Every traditional culture recognizes a role for the invisible, often translated with words like "spirit" or "life force", sometimes with more expressive terms like "angels", "demons", "gods" or even, simply, "God". It's tempting to dismiss these invisible forces as so much superstition, as though truth is made only of things we can see, but of course that's not quite true either. With the right instruments, we *can* see many invisible things; some of the greatest discoveries happen when a new gadget like a microscope or telescope makes people aware of a world that was previously hidden.

The invisible world of microbes is like that, with new, low-cost technologies showing us an incredible, rich, living universes of over 1 trillion species[1] waiting to be explored.

The word "microbe" refers to any tiny organism that carries its own genetic information for purposes of propagating itself. Far too small to see with the naked eye, dozens could fit inside a typical human cell. Although it's common to think of microbes synonymously with bacteria, in fact there are at least seven different types of microorganism:

- bacteria
- archaea: extremophile life forms that live and thrive in environments too challenging for bacteria
- protozoa
- algae

[1] Nobody knows for sure, but perhaps the latest, best estimate is Locey and Lennon (2016)

- fungi
- viruses
- a few multi-cellular animal parasites such as helminths.

Each of these has its own characteristic body type, means of reproduction, ways of moving around, and a deep, long history that is far older than humans.

Let's look next at some of these environments and see the odd places where microbes have been found.

5.1 Microbes above and below

Scientists studying a water-filled fracture two miles underground at the Mponeng gold mine near Johannesburg, South Africa, discovered *Candidatus Desulforudis audaxviator* by accident, after noticing odd levels of hydrogen compounds, by-products of the activity of an isolated bacterial colony.[2] Interestingly, this organism is a member of the same *Firmicutes* phylum that dominates human guts, though this particular bacterium evolved quite separately from us: it hasn't been exposed to surface water for millions of years. A systematic study of its genome revealed that, unlike other bacteria that usually live in co-dependent colonies, this one can survive all by itself, feeding on tiny bits of radioactive energy from uranium decay in an environment far removed from all other energy sources. It's not a great life: these creatures reproduce rarely, only once every few hundred or thousand years. But at least they don't have to worry about being consumed by predators down there.

Subglacial Lake Whillans is a lake buried under more than 800 meters of ice in the West Antarctic. A careful underground bore hole inserted by a team from Louisiana State University in 2014 found almost 4,000 different kinds of bacteria and archaea surviving under that ice.[3] The total bacterial count was not that different from what you'd find in surface lakes on other parts of the planet, a fact that is especially surprising for an environment that hasn't had a ray of light in millions of years. The bacteria instead thrive on iron, sulphur, and nitrogen as energy sources. [4]

Those may not be the deepest examples. A Cold War-era Soviet team drilling the world's deepest hole, were forced to abandon the project in 1994 at 12,261 meters (or 7.5 miles) underground, when they hit temperatures above 180 °C (or 356 °F), too hot for their equipment. Apparently the conditions weren't too hot for life, though: the nine-inch diameter Kola Superdeep Borehole [5] found 24 species of fossilized plankton among the two-billion-year-old rocks down there. Of course, fossils are not the same thing as living microbes, but even dead remnants at that depth is evidence of the tenacity of life.

[2] See Chivian et al. (2008)
[3] the WISSARD Science Team et al. (2014)
[4] http://earthsky.org/earth/diverse-microbes-found-deep-beneath-antarctic-ice-sheet
[5] http://www.atlasobscura.com/places/kola-superdeep-borehole

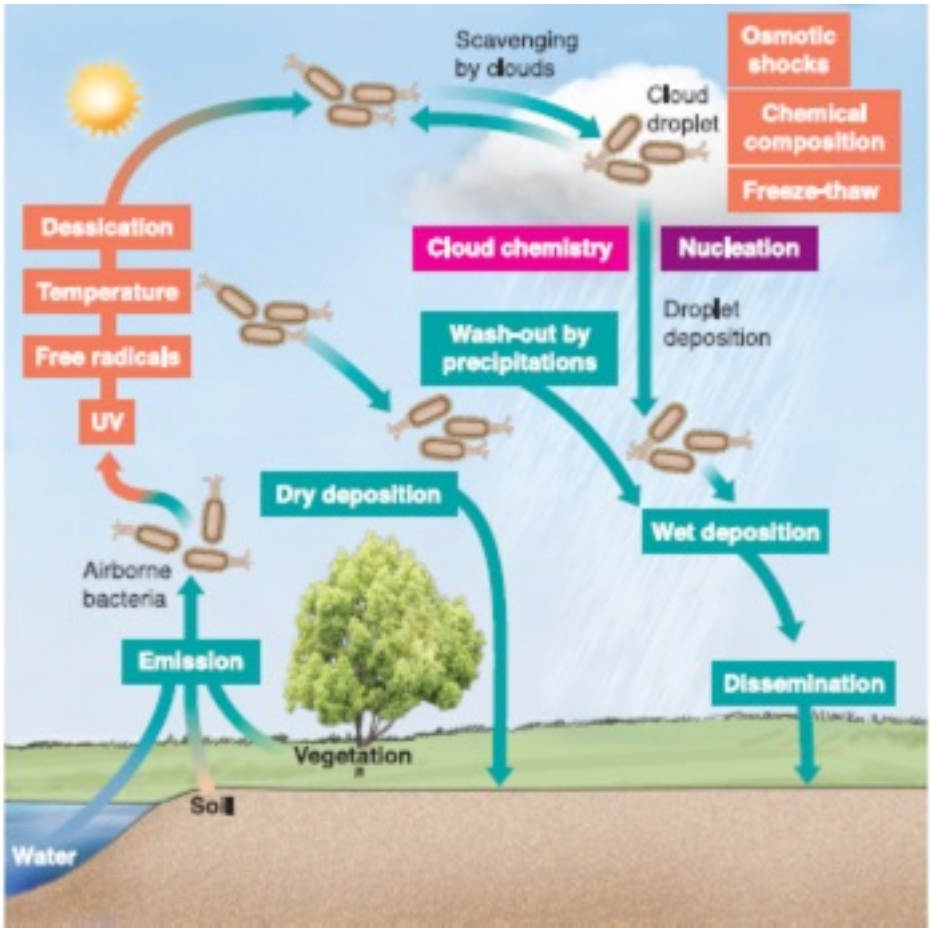
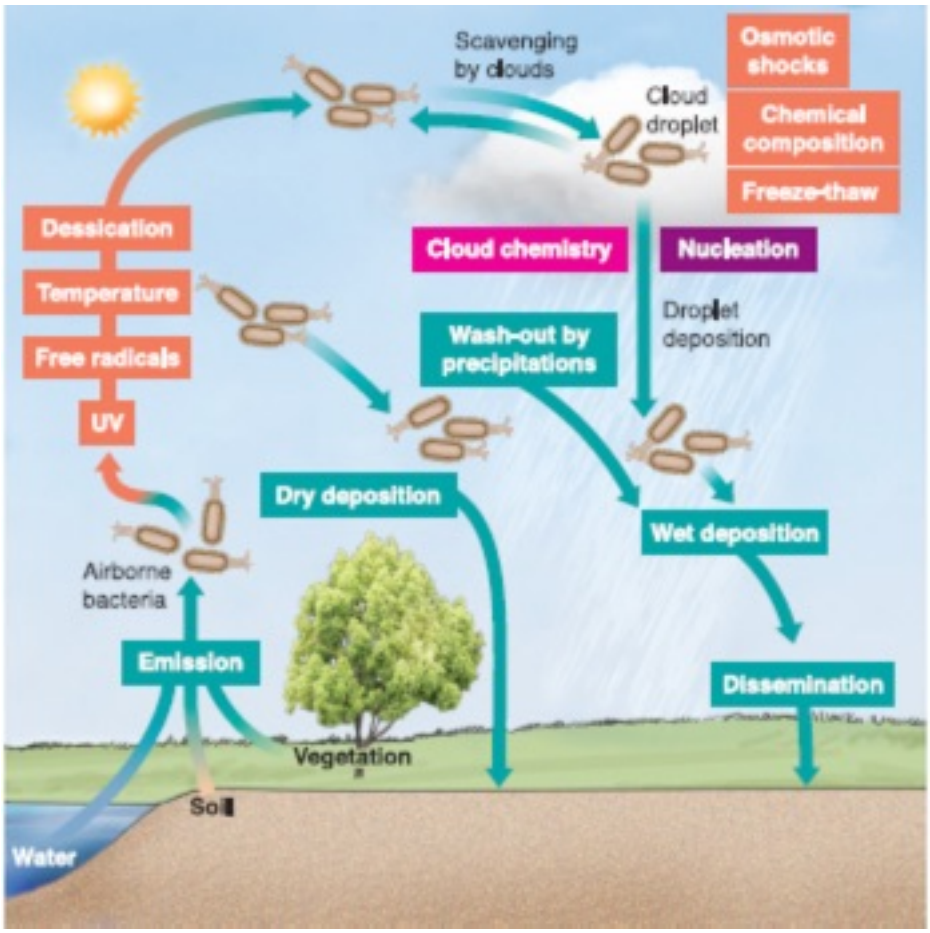


Figure 5.1: Go another 2,600 feet to find microbes. Photo: NASA/JPL-Caltech

Closer to the surface, a 2015 Chinese study[6] showed that 32% of the variety in an ecosystem is associated with variation in the life below ground, mostly bacteria that sustain the ability of roots to take nutrients out of the soil. Just knowing the temperature or precipitation levels of an environment won't tell you about the plants likely to be found there – the microbes matter too.

Even the sky contains living microbes. Scientists at the Institut de Chimie de Clermont-Ferrand in France have for decades sampled clouds to determine their precise contents, and sure enough: they find plenty of life there, usually between 1,000 and 10,000 bacterial cells per milliliter — not all that different from the amount you'd find in alpine snow. Like every living organism, these cells must soak up water and other nutrients, converting them into energy and various by-products, which collectively have a massive effect on the overall atmosphere, more than enough to affect climate. [7]



[6] Jing et al. (2015) and a summary here: https://macroecology.ku.dk/media/news_list/2015/09_biodiversity-belowground-is-just-as-important-as-aboveground/

[7] http://www.asmscience.org/content/journal/microbe/10.1128/microbe.7.119.1

source: ASMScience

The upper atmosphere is a harsh place for life: regular freezing and thawing, constant bombardment of UV radiation from the sun during the day, cold, subzero freezing temperatures at night, high speed, unpredictable winds that quickly disperse any colonies. Plus, at any moment these organisms can find themselves flushed to the ground in a rainstorm, where they'll need to adapt again.

These extreme conditions are just another day in the life for one species commonly found in clouds, *Pseudomonas syringae*, which harbors a protein in its cellular wall that reacts to cold temperatures, alternately preventing and allowing a water molecule to turn into ice and back. It doesn't take many of these reactions to generate precipitation. With so many cells constantly floating in the atmosphere, even small changes in concentration — perhaps due to human activity on the ground — can, at least theoretically, make the difference between rainfall and drought. How much of an effect is hard to say: you can imagine how difficult it is to study bacteria floating in the sky.

Our inability to access these environments is often the biggest reason we remain ignorant of the life that is found there, but there have been many attempts to learn more. Formal studies about the viability of microbes in space have been conducted since the early 1960s [8], when Apollo-era scientists wanted to understand the dangers of space travel, both to any humans in space as well as to those of us on the ground who might be exposed to any intersteller visitors.

Although new and bizarre extremophiles are discovered regularly, so far it appears that even the hardiest of known organisms have a tough time when directly exposed to solar ultraviolet radiation. But the particularly resilient spore-making *Bacillus subtilis*, for example, it is estimated could survive for at least six years if it were shielded somehow from direct sunlight, say embedded inside a meteorite.[9]

Several lichen species, including rock colonizing *Rhizocarpon geographicum* and *Xanthoria elegans*, and the vagrant *Aspicilia fruticulosa*, remained alive after ten days of direct UV exposure on board a European Space Agency spacecraft. [10] Some especially hardy cyanobacteria that came with the lichens didn't survive, so perhaps space offers a better chance for multicellular life, which has the luxury of outer protective pigmented layers.

[8] Hotchin et al. (1967)

[9] See an extensive 2010 review of everything known about space microbes in Horneck, Klaus, and Mancinelli (2010). Or skip to the handy summary table

[10] Torre et al. (2010)

Figure 5.2: Could be lichen outer space. Photo: J Brew

Traces of sea plankton, for example, have been found in space, on the surface of the International Space Station, where they are believed to have floated from the upper atmosphere. [11] Why?! How did they get there! Who knows!

What is known is that between a quarter and two-thirds of microbes in the air are entirely new and undiscovered organisms. A study of the "air microbiome" above New York City found bacteria and viruses that apparently originated in water, soil, vegetation, as well as in animals and humans, but even then few patterns emerge. Although there appear to be distinct microbial environments, on the land versus water, for example, overall many of these organisms are quite hardy and seem to find themselves migrating all over the place.

They can migrate in the smoke from a wildfire. A 2008 study by University of Idaho scientists[12] identified a dozen microbes that are transported in plumes of smoke, despite temperatures of

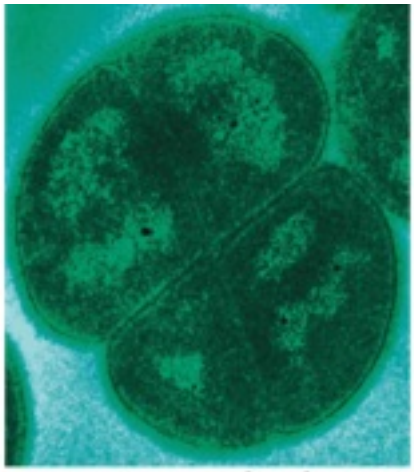
[11] http://tass.ru/en/non-political/745635
[12] Kobziar et al. (2018)

250º C or more. The new science of *Pyroaerobiology* is uncovering many examples where smoke-transported microbes have an impact on forest health and more.

Still other microbes thrive in radioactive environments, like the dangerous interior of a nuclear reactor. *Deinococcus radiodurans* is an extremophile member of Phylum Deinococcus-Thermus that boasts an impressive built-in DNA repair mechanism that lets it survive cold, vacuum, acid, light, dehydration – you name it. It remains unbothered by radiation levels more than 1,000 times higher than would kill a human.

Microbes seem capable of living off just about anything. *Ideonella sakaiensis*, discovered in 2016 by a Japanese team[13], can break down and metabolize plastic, just like the fungus *Aspergillus tubingensis*, found in 2018 in a garbage dump in Pakistan, which eats polyurethene in months rather than decades.[14] The waxworm *Plodia interpunctella*, observed eating plastic in a lab probably owes its digestive abilities to other, as-yet-to-be-studied microbes.

In fact, many non-microbial organisms owe their most defining characteristics to microbes. Termites wood-eating abilities are thanks to a whole community of synergistic bacteria, archaea, and protists. Aphids can't live off sap without *Buchnera*, a microbe that supplies them with essential amino acids. Some microbes even play a role in the mineralization of copper and gold.[15]



Deinococcus radiodurans

[13] http://www.sci-news.com/biology/ideonella-sakaiensis-bacterium-can-break-down-metabolize-plastic-03693.html
[14] Khan et al. (2017)
[15] Bütof et al. (2018)

5.2 Microbes around you

You don't have to go to extreme conditions to find unusual microbes. Microbes thrive whereever humans live, and they are in our everyday environment too. The PathoMap Project, studying DNA collected from the New York City area found that, like the air above, half of the microbes we walk past everyday are unknown to science. [16] Most of the organisms are apparently benign, with no obvious affect on humans one way or another. Even when known pathogens are found, including *Yersinia pestis* (Bubonic plague) and *Bacillus anthracis* (anthrax), the lack of reported infections indicates that probably these organisms are busying themselves for some other, unknown, and maybe even useful purpose[17]

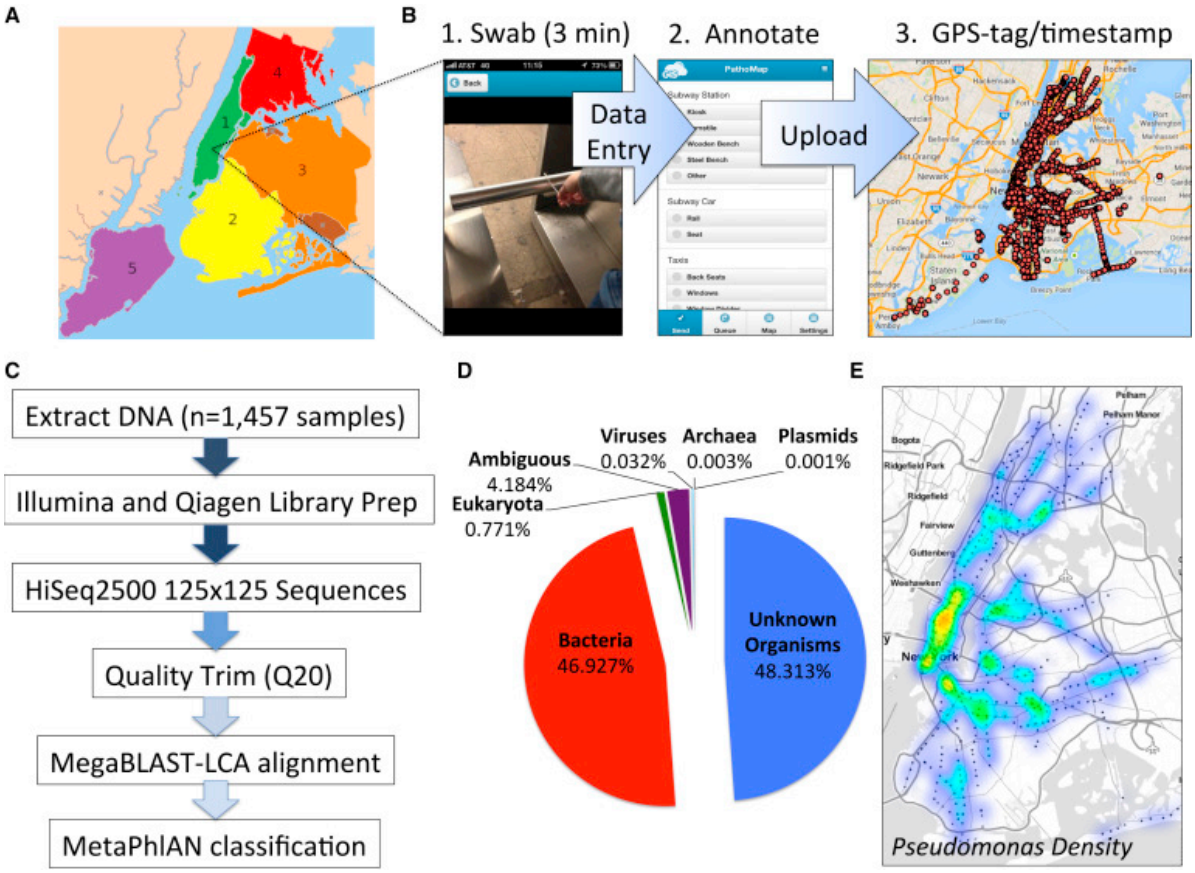


Figure 5.3: Half of the organisms collected by the Pathomap study are unknown. Source: Afshinnekoo et al. (2015)

[16] Afshinnekoo et al. (2015)

[17] A later, more careful analysis indicates these particular pathogens may not actually be present: http://msystems.asm.org/content/msys/1/3/e00050-16.full.pdf

Generally the microbes seem content to exist patiently with no apparent affect on the environment. A station flooded by Hurricane Sandy showed a similarity to a marine environment a year after the disaster.

Humans are the source of many unusual microbes in our environment. Regularly shedding 1.5 million skin cells per hour, your body's leftover inhabitants can colonize a hotel room in less than six hours.[18]

Your household pets carry microbes, of course, but simply having a pet seems associated with different microbes in humans. One study showed that babies living in a household with pets have more *Clostridiaceae*, *Veillonella* (especially for dogs), *Peptostreptococcaceae* and *Coprococcus*. Cats in particular seemed associated with lower *Bifidobacterium* while dogs seemed to spell doom for *Eggerthella*.[19]

[18] http://www.wsj.com/articles/big-data-and-bacteria-mapping-the-new-york-subways-dna-1423159629
[19] Azad et al. (2013)

6 Microbes and You

6.1 The promise and disappointment of genetic testing

If you want to optimize your health, you'll eventually need to understand more about your genes. Wearable devices like FitBit or Apple Watch, or a nutrition or dieting app like MyFitnessPal, can help optimize some aspects of your physical body but hard work and discipline will take you only so far. As you reach the limits of how much you can change, you'll settle into the discovery that the genetic component is undeniable. Over one million customers of the genetic testing company 23andme have opted to look at their genes in part to understand better what their own limits are.

Genes do seem important. Everything from twin studies to laboratory experiments with knock-out mice shows that large parts — perhaps the major part — of our health and even behaviors are determined as much by our genetic makeup as by the environment in which we put ourselves.

Still, despite much progress since the unveiling of the Human Genome Project in 2001, there are frustratingly few examples of genes that decisively determine one trait or another. Except for a few simple cases like eye or hair color, most genes seem merely to increase or decrease the odds one way or another. When you read the details about your own genes, you'll be disappointed at how little about genetic testing is truly insightful. Did you really need a DNA test to tell you that you are lactose intolerant?

Worse, even when the science tells you something you didn't know — your likelihood of Alzheimers or Grave's disease — there often isn't much you can do about it besides eat healthy and get plenty of exercise. In fact, with disappointingly few exceptions, nearly all conclusions you'll get with DNA results will be advice you should be doing anyway.

What's an optimizer to do? On the one hand, the evidence is powerful that genes determine much or most of your health, but on the other hand, you can't do much about it beyond the obvious. The results of human DNA testing just aren't all that actionable.

Fortunately, one of the most exciting consequences of the latest science on human genetics is the role played by other genes in your body. And the best news: you can change them! And you don't need a fancy laboratory with complicated equipment for recombinant DNA. This book will show you how, through experiments on the types of food you eat and deliberate changes you can make in your environment, you can make a significant difference in kinds and functions of the genes inside you.

6.2 Most of your DNA is not fixed

If you could zap me with a scanner that can break down everything in my body, all the physical "hardware" inside me right now, you'd find a curious fact: although it's true that 99% of the weight and size of what you see is human (blood, skin, bones, organs, etc.), only about half of the *cells* are human, and even less than that — perhaps as little as 1% — of the DNA-carrying genes are human.

What's the rest? Who am I, if only 1% of the genes inside me are human? The answer is microbes, and as befits something that so outnumbers the "human" part of us, they play a large role in everything about what we do, from our health to our moods and even to our motivations. To put this more precisely, humans have 20,000 - 25,000 genes, but just the microbes in your intestines alone have an estimated 2 to 4 million genes.

These microbes and the important DNA they carry are constantly changing, sometimes quite significantly, depending on what you eat, who you're with, and a host of other factors that you can manipulate.

6.3 Microbes are older than any of us

We tend to think of the invisible, single-celled microbes as "primitive", not nearly as "advanced" as we humans, with our marvelous brains and ability to transform the earth with airplanes and skyscrapers and nuclear reactors and all the rest. But that's what we would think, wouldn't we? In fact, the microbes are everywhere, literally everywhere on earth, in the sky, even deep underground. We can't go anywhere without encountering microbes because, well, there are even microbes on and inside us. Humans can't survive without microbes. So what does it even mean to say we're "better" or more "advanced" than they are?

Before the microscope, people didn't even know that microbes existed. Similarly, until the advent of large-scale gene sequencing machines in the past ten years, almost nothing was known about the amazing ubiquity and resilience of microbes. Yes, they're single-celled and yes many of their cellular functions seem more straightforward than the functions of a multi-cellular creature like us, but it would be a mistake to assume that means microbes – collectively – are less influential, and certainly it would be a big error to assume they are less important. Fact is, these organisms have been around, breathing, eating, multiplying, for billions of years, often in pretty much the same form that they are today. These things have survived every imaginable planetary condition from volcanoes to the depths of the ocean to the inside of nuclear reactors. Global Warming means nothing to these guys, who have seen and thrived all over the earth since the day life first appeared.

6.4 What they want

Because they have such a huge advantage over us, in lifespan (each microbe is an exact divided copy of itself, going back a zillion generations), in speed of replication (they can double in just a few minutes under the right conditions), and ubiquity (as I said, cellwise they far outnumber us), they can afford to colonize every new imaginable environment.

And that's what they do, every time a new frontier opens to them. The moment of your birth, for example, when you left the (mostly) sterile conditions of your mother's womb, they immediately flooded all over your skin, mostly coming from your mother, and in that fresh environment they used their first-mover advantage to get a stronghold that in many cases lasts your entire life. Many (most?) of the microbes that matter arrived inside you this way, originally, and many of them are still there today, decades, even half a century or more later.

To survive, they need one thing: something to eat. Being so tiny, they don't need much, and they mostly eat things that you (and other larger creatures) weren't interested in anyway. (Or, since they were here first, it's probably more accurate to say that you and I must live on the foods that they don't want. A cheeseburger is only food for you because you snatch it faster than they do. Leave it outside for a while and they'll get it eventually).

6.5 Who's in charge?

Collectively, the multitude species of microbes will eat just about everything, but individually each species has its preferences. When they're outside the body, as I said, they can "colonize" new territories (like fresh baby skin) to get what they want, but those inside your tummy are at the mercy of whatever it is you decide to put into your mouth.

Often, that's not a big deal: many species thrive on the same proteins, carbohydrates, and fats that you do. But some species do better than others with certain types of foods, and this is where the line between your human desires and theirs becomes unclear.

Eighty percent of all your brain's outside receptors – counting all the nerve endings everywhere on your skin – eighty percent complete their connections in the gut. The main switching grounds, an area called the vagus nerve, does *something*. What? We know very little, but we see some evidence that the purpose – the reason that not 1% or 10% or 50% but a full 80% of all the receptors go to the gut – is so the microbes can tell your brain what to do.

When you find yourself feeling hungry, ask yourself who is feeling hungry. Scientists have traced that feeling of hunger to changes in certain hormones like leptin, but wait – why did the leptin levels change in the first place? Could it be that a microbe someplace was manipulating your leptin levels, perhaps by poking that vagus nerve just the right way – to get your brain to start thinking about whatever food that microbe wants?

This isn't as ridiculous as it sounds, the idea that microbes could influence your feelings and desires. Think about a disease like the rabies. Because it spreads through saliva, it can't find new territory unless its host somehow finds itself exchanging saliva – biting – another potential host. So guess what a rabies victim can't stop thinking about? Biting a new victim. The microbe literally puts a thought into the mind.

There are many other examples, so many in fact as to be potentially a bit disturbing when we realize that we humans may be much more at the mercy of tiny microbes than we think. Links have been made between microbes and schizophrenia, stress, anxiety, self-grooming, and much more. Autism Spectral Disorders, which have always seemed puzzling because of the relationship they seem to have with digestive problems, are also linked to microbes, or the lack thereof.

Perhaps the most intriguing example is the common parasite *Toxoplasma gondii*, the strange organism that can only reproduce in the intestines of cats. A parasite seen often in all warm-blooded mammals, it's found in about a third of the global population of humans too. It's one of the reasons they tell pregnant women to stay away from cat litter. But here's the interesting part: when a Toxo protozoa infects a mouse, it leaves cysts in the mouse brain that make it attracted to cat urine! Yes, it changes the neurology of a mouse so that it's more likely to end up inside a cat's tummy – exactly where it can reproduce.

Think about this too much, and you'll end up with the obvious question: what other weird microbes are infecting us right now? Can we explain some of our own behaviors this way? Is there a human equivalent of these infections, driving us to do things we "ordinarily" wouldn't do? And maybe these microbes are so ubiquitous, teeming all over us and in our brains, maybe there's no way to even know what "ordinary" or "normal" human behavior is.

6.6 What is health?

Modern, western medicine tends to think reductively about health, dividing the body into pieces like organs and cells and prescribing interventions that target one particular aspect of the whole, with specific drugs or supplements. But of course nothing as complex as the body and health can be simplified this much. Maybe you can't really think about human hardware without thinking about the whole ecology that goes with it, the various organisms live in and around us and greatly outnumber us.

From this perspective, the whole idea of "health" takes on a new meaning, because we're no longer talking about the status of a single organism – me – but rather about the entire functioning ecosystem of many, many living things, including the "me" that I want to refer to as a human. You can't survive long without these microbes any more than you can survive without air. This whole "me", sometimes referred to as the "holobiont", is perhaps the true unit of what it means to be human and healthy.

Redefining health in terms of the holobiont has important implications for treatment. If it's the *entire* ecology in and around me, then targeting a single unit or a single symptom may not be the best solution. Treating a skin condition with an antimicrobial salve, for example, may inadvertently destroy other microbes necessary for some other function. Teeth-brushing or hair shampooing, while seemingly fundamental aspects of hygiene, may not be simply about "getting rid" of something that we think of as "bad", such as an unwanted odor. In your zeal to rid yourself of one thing (the odor) you may be introducing another (a skin condition someplace else). It might be better to treat the root cause, figure out why the odor is there in the first place.

But what is a "root" cause in a complex ecological system like our bodies? After all, anything that affects one part of the body is likely to affect others as well. Is there a way to affect everything all at once?

Diet is one way. What you eat is an input to the entire ecosystem.

Where you live – your environment – is another. From the air you breathe (is it clean? cold?) to the amount of stress you face, change your immediate surroundings and you will change the ecosystem.

If we no longer think of our bodies as independent parts, then our treatment options must be holistic. No intervention should be done without considering the consequences it has on the whole. Similarly, it may often (usually?) be true that the best treatments are dietary or environmental – facing the entire holobiont at once.

7 Microbes In You

7.1 Microbes and Disease

The great French scientist Louis Pasteur, working as a physician in the 1800s, was the first to popularize the idea that the world is covered in *germs*, invisible agents that he associated with food spoilage and disease. Simple steps at hygiene (the word derives from a Greek phrase meaning "healthful art") could make conditions inhospitable for them, he discovered, enabling better food storage and dramatically fewer illnesses. Working as a chemistry professor in the 1850s, one of his students, the son of a local wine manufacturer, sought his help to solve problems with souring. Subsequent investigations led him to conclude that invisible yeasts were the culprit, and that exposure to air could affect the rate of fermentation. Pasteur's emphasis on controlling these microbes led to a general association of germs as pathogens, a bad, even evil force that we must destroy, every single time. The only good germ is a dead germ.

And for good reason. The development of effective disinfectants, and then antibiotics – germ killers – was one of the greatest medical achievements of all time, saving the lives of a significant fraction of the human race. Before Pasteur, urban life was a precarious game of chance against diseases that seemed to come and go randomly. Thanks to the development of the Germ Theory of Disease, it was suddenly possible to imagine a world where deadly illnesses and infections could be controlled and perhaps eliminated. Now, every day of your life, modern amenities like running water and flush toilets keep you healthy simply by controlling the growth of microbes.

Some can be quite nasty. The bacterium that causes Cholera, *Vibrio cholerae*, after infecting the small intestine, promptly hijacks the body's natural defense systems into sending a large stream of water through the colon to flush out all other bacteria. Normally, this would be an appropriate response to an invasion, but by hiding before the colon, *Vibrio cholerae* continues to breed above the main flow of water. The resulting diarrhea is so fierce that the patient is literally unable to drink enough to make up for the outflow, and dies of dehydration within days. And in a final act of cruelty, that water that the body pushes out so fiercely is itself full of *V. cholerae*, who use the opportunity to infect others who come in contact with the water.

Bacterial gastroenteritis – more commonly known as "food poisoning" – is a source of discomfort, abdominal pain, diarrhea, and worse for about 1.5 million Americans each year. Most of the time, it can be traced to *Campylobacter jejuni*, which has a shape and structure ideally

suited to penetrating the mucosal layer of your intestines, where it attaches itself and begins to release toxins that activate the immune system and the resulting diarrhea and fever. *C. jejuni* is a natural and benign colonizer of the digestive tracts of many bird species, including poultry, and because most of the time these birds appear perfectly healthy, it's not uncommon for 20% or more of retail chickens to be contaminated. Fewer than 1,000 organisms under the right conditions can cause illness.

Fortunately, *C. jejuni* is easy to kill. Low pH, for example: 2.3 and they're dead (think lemon juice or vinegar). The antibiotic erythromycin is quite effective too, with almost no resistance observed so far. But the best weapon is heat: they strongly prefer the normal body temperature of birds (40° C or 105°F), and reproduce best at 42° (107.5°F). Go much higher than that and they'll slow down and die.

The Limits of Bacterial Reproduction

Pathogenic foodborne bacteria stop reproducing below a certain minimum temperature and above a certain maximum temperature—and replicate fastest within an optimal temperature range. The acidity, or pH, of the food also places limits on bacterial multiplication.

	Lower temp. limit		Upper temp. limit		Fastest growth		Lower pH limit	Upper pH limit
Species	(°C)	(°F)	(°C)	(°F)	(°C)	(°F)	(pH)	(pH)
Bacillus cereus	4	39	55	131	28–40	82–104	4.3	9.3
Campylobacter jejuni	30	86	45	113	37–43	99–109	4.9	9.5
Clostridium botulinum Type A	10	50	48	119	30–40	86–104	4.6	9
C. botulinum Type B	10	50	48	119	30–40	86–104	4.6	9
C. botulinum Type E	3	38	45	113	25–37	77–99	5	9
C. perfringens	10	50	52	126	43–47	109–117	5	9
Escherichia coli (pathogenic)	6	43	50	121	35–40	95–104	4	9
Listeria monocytogenes	–1	31	45	113	30–37	86–99	4.4	9.4
Salmonella spp.	5	41	47	116	35–37	95–99	3.7	9.5
Shigella spp.	6	43	48	117	37	99	4.8	9.3
Staphylococcus aureus	7	44	50	122	35–40	95–104	4	10
Vibrio cholerae	10	50	43	110	37	99	5	10
Yersinia enterocolitica	–2	29	42	108	28–30	82–86	4.2	10

Figure 7.1: How to kill common pathogens. Source: Myhrvold et al. (2011) p.145

The toxins produced by the *Clostridium* genus are among the most dangerous. Botulism (C. *Botulinin*) Tetanus (*C. tetani*), gangrene (*C. perfringens*), and of course C. *Dificile.*

Some bacteria simply use the darkness and wet warmth of the colon as a breeding ground, happily feasting on the materials they find there. They cause trouble not by what they eat, but by what they excrete: nasty toxins that mess up some other part of the body. *Clostridium*

botulinum produces the neurotoxic protein botulinum that can weaken or freeze nerve cells. The most acutely lethal toxin known – only 2 billionths of a gram can kill – botulinum is almost as deadly to people as the plutonium in a nuclear bomb. Just a few pounds under the right conditions would kill everyone on earth.

Part of what makes pathogens so dangerous is it takes so few of them to be deadly. Some *Shigella* species, for example, become infectious with a starter colony of as few as ten organisms.[1]

With such terrible killers lurking out there, it's tempting to divide all microbes into "bad" (pathogenic), "good" (probiotic) and "neutral" (commensal). You'll find plenty of lists that do just that.

But often, perhaps usually, the distinction between good and bad is unclear. Consider the "viridans" *Streptococci*, a group name for a whole breed of related microbes commonly found harmlessly in human mouths. If a few of these escape the mouth and somehow enter the bloodstream, they can land on a heart valve and can cause a dangerous, life-threatening infection. But inside the mouth they are tough competitors to other bacteria that may want a foothold, like the *Streptococcus* that causes Strep Throat. Mix Viridans with Strep A, and Viridans wins every time. So is it good or bad? Well, it's bad if your Viridans makes it to the heart; but in its regular form it protects you from other infections.[2]

People with *Streptococcus lugdunensis* in their noses appear protected from some staph infections, probably because *S. Lugdunensis* produces a microbial antibiotic to kill off its competitors. But *S. lugdunensis* can itself cause skin infections.

Often it's the context that matters. *Staphylococcus aureus*, found in about a quarter of all Americans, is the agent behind a host of infections ranging from mild skin ailments to the deadly, often untreatable MRSA. But it seems to be harmless when in the presence of *Corynebacterium* species. [3]

Fortunately, the body is pretty good at fighting off many pathogens. Around 94% of people who ingest *Salmonella* will recover without any medical attention at all.[4] Sometimes the fight against bad bugs is helped by other bugs: *Lactobacillus* for example is especially good at crowding out pathogens.

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[1] Kothary and Babu (2001)

[2] see page 119 Blaser (2015)

[3] https://www.sciencedaily.com/releases/2016/08/160817091034.htm

[4] Of course, it's difficult to tell how many people *don't* go to the hospital; this is a risk estimate based on fairly generous assumptions about the amount of the pathogen in eggs and how many people eat them. Hope et al. (2002)

Members of your household will have more similar microbiomes if there's a dog present.[5]

Humans are able to synthesize just 30 plant-digesting enzymes. Contrast that to the species *Bacteroidetes thetaiotaomicron* which can break down plant structures using over 260 different enzymes.[6]

7.2 Gluten

It's been well-established that a gluten free diet impacts the microbiome. This shouldn't be too surprising, given that gluten is a nutrient for some bacteria but not others. But what about people who show an unusual sensitivity, even allergy, to gluten? What's the cause?

Recently the idea of a gluten free diet has taken on fad diet status. Despite surprisingly little research evidence that it can quantitatively affect health, millions of people swear that gluten gives them various ailments from poor digestion to brain fog. If you don't believe it, they'll say, try it yourself and see; and sure enough, many of those who go off gluten claim big health benefits. Eating is usually a zero-sum game: stop eating one thing (say, the gluten in wheat) and you'll end up eating more of something else (rice or corn). Is it the switch to a different diet — and the anticipation of success that this brings — that makes people feel better, or is there something really significant about gluten itself?

The experts say no, with one important exception. A tiny fraction of people *do* suffer from Celiac disease, a known disorder of the body's ability to handle gluten. There are well-established tests that can definitely tell whether you have Celiac disease or not, and although the vast majority of people test negative, those who are true Celiacs will immediately and obviously benefit from a gluten free diet. But what's driving the difference?

As usual, the genetic evidence isn't completely lock-tight. Although a third of the population have particular versions (DQ2 or DQ8) of the cellular receptor human leukocyte antigen (HLA), only a tiny minority go on to develop serious gluten sensitivities. Some studies indicate gluten sensitivity arises at an early age, and that perhaps celiac disease can be prevented by exposing babies to gluten at just the right moment, but other studies say the opposite.[7]

These are all clues that the microbiome may be involved, and sure enough, many studies show a definite difference between healthy microbiomes and those with clinically-proven Celiac disease.[8] But because Celiac sufferers tend to eat differently than non-sufferers, it can be hard to tell how much of the microbiome is a result of a different diet, and how much is due to the disorder.

[5] see Song et al. (2013) or open text
[6] see Spector (2016) p.299
[7] Vriezinga et al. (2014)
[8] De Palma et al. (2009)

Another clue happens further up the digestive system. Spanish researchers looking at the small intestine found curiously similar microbiomes in both healthy and celiac patients. [9]. The difference happened at the functional level of what those bacteria *do*, and not necessarily in just whether the microbe is present or absent. *Lactobacillus*, it turns out, is one of the best degraders of gluten[10], but there are others: *Bacillus pumilus*, *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa*. Most interestingly, unlike *Lactobacillus*, some of these bacteria can do more than just digest the gluten: they appear to contain enzymes that transform the gluten — pointing to the possibility that the enzymes produced by these microbes could be purified and used to eliminate traces of gluten from food products.

So if Celiac disease is caused by a change in the way some microbes function, how did that change happen in the first place? One theory is that it's a virus. Researchers at the University of Chicago and University of Pittsburgh were able to supercharge the way mice react to gluten by infecting them with a reovirus that apparently changes something about the body's immune response in the presence of the gluten protein. [11]

7.3 Diversity and health

Most microbiome discussions begin with the assumption that diversity is good. Virtually any popular book or article about how to improve your health will suggest ways to increase diversity, usually by eating specific foods. For what it's worth, a study of more than 10,000 gut microbiomes found only one sure-fire association with higher gut diversity: people who self-report eating more plants have higher diversity than those who eat fewer types of plants, and this is true no matter their diet type (omnivore, vegan).[12]

The intuition is easy to understand: if your body harbors a wide variety of microbes, you'll have a deeper catalog of useful ones that can be applied to new situations. The world around us is constantly changing, and you never know what new threats or opportunities you may encounter. You can respond better if you have an abundant variety of organisms that can meet any challenge.

In practice, diversity is difficult to pin down quantitatively. We know what we mean in principle: having a variety of different microbes seems good, but clearly there are limits. You wouldn't want "variety" to include serious pathogens, for example. We know intuitively that a deciduous forest at sea level, with dozens of differerent tree species, is more diverse than one at a high altitude tree line. But is the one at low altitudes "better"? It depends on where you live!

[9] Herrán et al. (2017)
[10] Rizzello et al. (2007)
[11] http://www.today.com/health/celiac-disease-may-be-caused-virus-new-study-finds-t110119
[12] McDonald et al. (2018)

Table 7.1: Diversity example: two forests with an equal number of trees, and one with fewer trees.

Forest	Number of Trees	Number of Species	Diversity
A	1000	1	Low
B	1000	1000	High
C	10	10	?

A similar problem has long confronted ecologists, who have developed several diversity measures that have been adapted to the micro world:

- **Alpha diversity**: the variance *within* a particular sample. Usually measured as a single number from 0 (no diversity) to infinity, or sometimes as a percentile, this is what most of us mean when we look at our microbiome results and ask about diversity.
- **Beta diversity**: how samples vary against each other. Many scientific studies are interested in the differences between sites on the body, or microbiomes across geographic locations. Beta diversity is typically the thinking behind "clustering" algorithms that try to show differences or similarities among samples.

All diversity metrics take into account two aspects of a community: the number of different organisms in a sample, and the range of abundances for each one. To understand how this works, think of two forests, each with an equal number of trees. (Table 7.1)

Clearly, Forest B with its abundance of species and trees is the most diverse. But what about Forest A compared to Forest C?

On the one hand, Forest C seems to have a greater variety of trees: 10 times more than Forest A. But it also has many fewer of them. In other words, there are two aspects of diversity that matter: the absolute number of organisms in an ecosystems, and the variety or *richness* of those that are there.

Whether A is "better" or "worse" than C depends on subjective, non-quantifiable factors that are not included in any diversity metric. A managed forest, such as one on a Christmas tree farm, might be perfectly healthy for one purpose (growing Christmas trees for sale), while an adjacent clear-cut forest with ten lonely and scraggly trees could be far less healthy, even if it has more of a variety of trees.

In this example, we use the distinction *richness* to refer to Forests B, or C, with their greater variety of species, and the overall term *diversity* tries to be a measure of *both* richness and abundance.

We can apply the same principle to our taxonomy tables: A microbiome sample with 100 unique taxa is more diverse than one with only 10 unique taxa. But if we just use raw, absolute numbers, it can be hard to compare across different microbiome tests. For example, what if I have two samples, each with 100 unique taxa, but in one sample there are tiny

amounts of all but one of the taxa, while the other sample has equal amounts of everything? Which is more diverse?

One way to quantify this is with a metric borrowed from probability theory. What if, instead of looking at *all* the taxa and their respective amounts, we simply take at random any two taxa from the sample: what is the probability that the two will be the same?

If I have a sample with 100 unique taxa, each of identical abundance, then the odds are pretty low that I would select at random two of the same taxa; conversely, if a majority of the sample consists of the same taxa, with many other taxa of smaller abundance, then the odds are pretty good that the two I select would be the same.

In fact this is generally the case in healthy western guts, which are usually composed of only two large phyla: *Firmicutes* and *Bacteroidetes*. In my case, as you'll see, these two phyla make up over 90% of everything in my samples; the third most abundant taxa rarely breaks 10%. The odds that you would randomly pick these two is pretty high. That's the intuition behind the Simpson metric, developed in 1949 by the British scientist E.H. Simpson.

But note that with Simpson, *high* numbers mean *low* diversity; after all, in a homogeneous sample with no diversity, the odds that you'll pick the same taxa will be 100%. To keep this consistent with the idea that higher numbers mean *more* diversity, most scientific studies of the microbiome use *Inverse Simpson*, which is simply 1 divided by the Simpson number. Note that for very low Simpson numbers, the Inverse Simpson value can be quite high, even approaching infinity when dealing with a microbiome with many unique and extremely low abundance taxa.

The taxonomy of microbes matters too. Each successively lower taxonomical rank always has at least as many taxa as the higher levels, so you can't simply count the total number of taxa at a rank. A single genus like *Bifidobacterium*, for example, can have dozens of species associated with it. For this reason, microbiologists usually measure diversity at the Family level: it's a good compromise between overall coverage and specificity of taxa.

In the real world, the type and variety of microbes in the body are constantly changing, so it's important not to get too hung up on a single number for a single sample. You'll see this later when we look at how diversity changes in my own experiments

The key is to take multiple samples and not rely on a single day's measure. If you take many samples over time, you'll find that the moving average is much more stable, and a better overall indicator of diversity.

There are other measures of diversity as well. The *Shannon Index* borrows from Information Theory to ask how much unique information is contained in a given sample. A radio signal that broadcasts random static, for example, would have a lower Shannon number than one for a music concert. Similarly, a microbiome with a boring makeup – all the same species, for example – would have a lower Shannon number than one containing a rich abundance of many different types of microbes. In practice, *Shannon* and *Inverse Simpson* tend to track one another reasonably well, a clue that they are getting at a similar idea. (Figure 7.2)

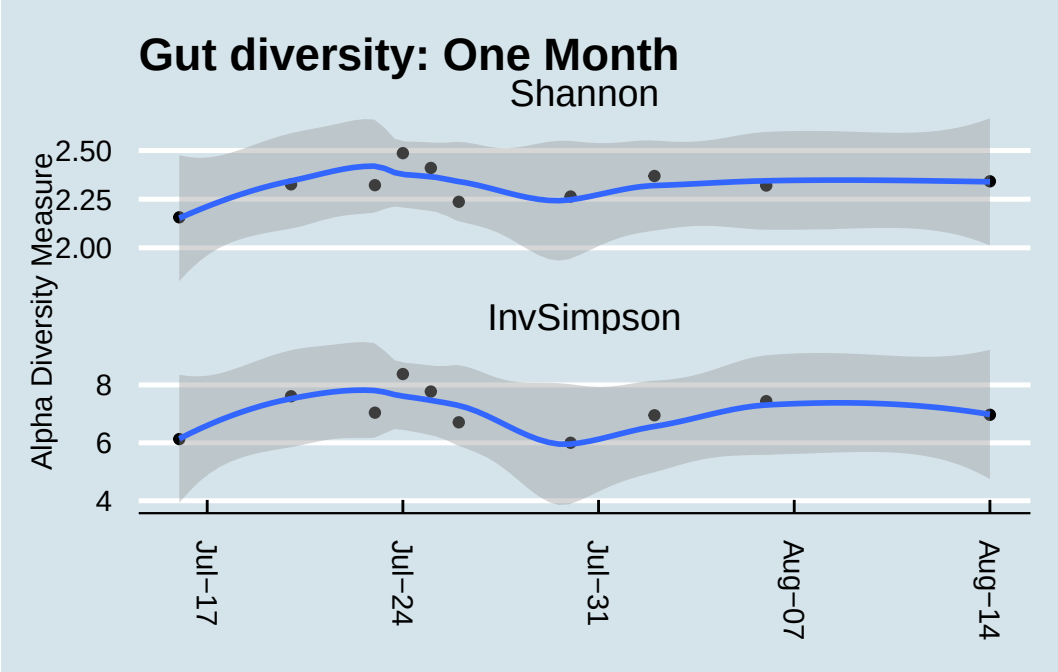

Figure 7.2: Comparing two types of diversity.

That said, Shannon tends to fall within a narrower, more predictable range, so I prefer it over Inverse Simpson when looking at my own samples. It often doesn't matter which metric you use, though, as long as you're consistent.

Nevertheless, I have learned to not place much stock in any diversity measure. After all, whether diversity is "good" or "bad" depends on *what* is in the sample. Is high diversity good even if it includes many known pathogens? Is "low" diversity good if it *only* includes one or two known commensal bacteria? As always in the microbiome world, it's hard to tell[13]

[13]See Shade (2017) for an excellent discussion of why diversity is generally a poor metric.

8 Microbes and Health

8.1 Food Allergies

If you attended elementary school before the 1980s, you can remember a time before nut allergies, when school lunches served peanut butter to everyone without the slightest worry that it might cause problems. Now many schools are forced to strictly limit the allowable kinds of food in their cafeterias, even from kids who bring their own lunches. Besides nuts, people suffer from allergies to milk, corn, eggs, fish, shellfish, soy, wheat, and many others.

Oddly, the very existence of food allergies appears to be an entirely modern problem. Medical journals didn't even mention food allergies until 1969, and examples were extremely rare before that. In fact, the very first mention of a food allergy happened about 100 years ago.[1]. Human genetics hasn't changed suddenly in the past half-century, and given the variety of conditions, it seems unlikely that we can blame it on a single toxin or industrial pollutant.

Several intriguing clues point in the direction of microbes as the cause of allergies. One theory, known as the "hygiene hypothesis", says that our modern environments are too sterile, that the immune systems of growing children need to be challenged by threatening invaders from time to time or they become overly sensitive. Now another idea, the "old friends" hypothesis suggests that it's not the hygienic conditions and lack of microbes *per se* that drive the autoimmune response, but rather it's that our bodies have evolved, over untold generations, to *expect* microbes in the environment, some nasty and some friendly, and when the developing immune system of a child is never exposed to these microbes, a breakdown occurs that misrecognizes certain foods as enemies instead.

Rutgers University scientist Martin Blaser (Blaser 2015) thinks something odd has happened because of the overuse of antibiotics. There are plenty of people in Western societies who suffer far fewer food allergies, people like the Old Order Amish of Pennsylvania, who for centuries have kept to traditional ways of farming and who live in communities largely unaffected by industrialization. Their significantly greater time spent outdoors, challenged regularly by animal and soil microbial pathogens has made them far less likely to suffer from allergies than the rest of the population. Studies of Amish gut microbiomes show strikingly different profiles, generally with higher diversity and numerous microbes rarely found in urban people[2].

[1] as mentioned in Spector (2016) referring to Golbert, T.M., J Allergy (Aug 1969); 44(2): 96–107. *Systemic allergic reactions to ingested antigens* and Schloss, O., Arch Paed (1912); 29: 219. *A case of food allergy*

[2] Zupancic et al. (2012)

Asthma, another terrible condition likely sparked by an incorrect balance between hygiene and microbes, points to better times ahead, its numbers of sufferers having peaked in the 1990s and 2000s. In fact, "We have probably seen the worst of the asthma epidemic"[3] writes scientist Tim Spector, who suggests childhood asthma has been replaced by food allergies.

The good news is that, armed with our understanding of the relationship between microbes and allergies, new discoveries may offer treatments or even cures.

Evidence showing that *Clostridia* may counter sensitivity to peanuts [4] has led to additional research behind the source of the problem. Now a new drug, Palforzia, is an FDA-approved treatment option for peanut allegies that works by exposing young children to small doses of the key microbe-stimulating compound in peanuts, letting immature immune systems develop a safer relationship with peanuts. Similar studies are underway for other allergies, lending hope that someday food allergies may once again fade into the background and disappear as they did 100 years ago.

Cathryn Nagler's lab has identified *Anaerostipes caccae* as a key microbe that protects against allergic reactions.[5]

8.2 Obesity

Obese and diabetic people are subject to more infections than healthy people, but interestingly it's not the body mass index that drives this, but rather the accompanying hyperglycemia.[6]

8.3 Hygiene

THIS CHAPTER IS STILL IN DRAFT

Microbiome diversity protects against pathogens by nutrient blocking : "Colonization resistance" is an ecological phenomenon. A diverse variety of microbes working together can hold back pathogens by soaking up nutrients.

Now for some speculation. I can't prove any of this, but these are some questions possibly worth asking:

What does (underarm) deoderant do? Although rich people soaked themselves with various colognes for thousands of years, the widespread use of deoderant is less than 100 years old.

[3] Spector (2016)
[4] http://www.sciencemag.org/news/2014/08/gut-microbe-stops-food-allergies
[5] Feehley et al. (2019) (download the full text here: https://cpb-us-w2.wpmucdn.com/voices.uchicago.edu/dist/e/1480/files/2019/07/Feehleyetal.pdf)
[6] Thaiss (2018)

Why do you have sweat glands there in the first place? Sweat is supposed to help cool your body, but not much cooling will happen from a surface that's not exposed to much air. Instead, those glands feed bacteria, especially the *Actinobacteria*, including *Corynebacterium* that generate those molecules that you can smell. [7]

Similar to the way some people do fecal transplants to modify their gut bacteria, someday it may be possible to do arm pit bacteria transplants.

How often should you brush your teeth?

Washing your hands in a public restroom

In Westernized countries for the past hundred years, it's been taken for granted that everyone should wash their hands every time they use the toilet, especially in a public place. Washing with soap and water, of course, is an effective way to remove harmful microbes, but I wonder what happens to your hands *after* washing, when you turn off the faucet or help yourself to the paper towel machine. Even in a restroom with those fancy automatic on/off devices, you still probably touch the doorknob on your way out.

We know that fecal matter often harbors pathogenic microbes, and of course the act of doing your business makes it likely that your hands will come closer to unpleasant waste products, but I wonder: most of the microbes you're touching are already on your body. I suspect that microbes optimised for some parts of your body won't do well on the hands anyway. But meanwhile, that faucet is being handled all day by crowds of strangers, each hosting microbes that probably enjoy the moist, pleasant surfaces of the bathroom sink.

Isn't it better to enter and leave the public restroom without touching anything other than yourself?

If you *do* wash your hands in the restroom, what's the best way to dry them afterwards? The short answer: paper towel: the friction from rubbing your hands helps loosen and dislodge microbes, while an air dryer can just spread more germs into the air. If you're concerned about the environment from all that paper towel waste, a careful analysis of the tradeoffs shows that both methods are about equivalent once you take into account manufacturing, installation, electricity, and final disposal costs.

How to wash food in the kitchen

By far the most common source of pathogens in food happens during meal preparation. This makes it tempting to wash everything over and over throughout the cooking process, but be careful: you might just be splashing those microbes onto other surfaces throughout the kitchen.

If you wash a whole chicken in your sink, for example, any germs on the exterior will unavoidably find themselves in tiny water molecules that may land anywhere in the kitchen. You'll

[7] see Callewaert et al. (2014) and http://www.realclearscience.com/blog/2014/08/antiperspirants_alter_your_armpit_bacteria_ar

scrub the counters afterwards, of course, but beware that it's hard to disinfect everywhere: the curtains? the ceiling?

Like most battles with microbes, there's a tradeoff between killing large numbers in a visible way ("carpet-bombing") and preventing them from gathering in volumes in the first place.

Generally it's best to leave food untouched if you'll be cooking it. Fruits and vegetables meant for eating raw should be cleaned, but just rinsing with water isn't going to dislodge the serious pathogens. Rinsing in vinegar or other acidic chemicals can kill most of the germs, at the expense of some flavor.

Ultimately the only sure way to beat pathogens in the kitchen is through high heat, so if you have reason to suspect that your food might be contaminated, you should cook it.

The dangers of kitchen sponges

A widely publicized 2017 study claimed that one of the most deadly microbial reservoirs in your kitchen might be the commmon scrub sponge. The porous nature of a sponge, the researchers claimed, let harmful microbes hide and breed, even after running them through a hot dishwasher or microwave oven. But a closer look shows the study used only 17 sponges, and the microbes found were all harmless, generally found on skin (as I confirmed myself in my Skin Experiments.

Microbes and cooking

Few microbes that inhabit human bodies are able to survive long at temperatures over 165 degrees (F).

8.4 Cancer

8.4.1 A microbiome-based cancer diagnostic

An all-star team from the highly-regarded Rob Knight lab at UCSD published in *Nature* (2020) a ground-breaking study that showed how cancer could be diagnosed through microbiome testing.[8], but a team of Johns-Hopkins statisticians (Gihawi et al. 2023) showed that there were several errors in the study. Knight's team did a reanalysis and claims that a later replication indicates that an association exists whether it's those particular microbes involved or not.[9]

[8] Poore et al. (2020), later retracted
[9] see *Science* 2-Aug-2023

8.4.2 Targeting microbiota in cancer

see April 2022 Targeting the gut and tumor microbiota in cancer

Park, E.M., Chelvanambi, M., Bhutiani, N. et al. Targeting the gut and tumor microbiota in cancer. Nat Med 28, 690–703 (2022). https://doi.org/10.1038/s41591-022-01779-2

Eric Topol summary (Evernote)

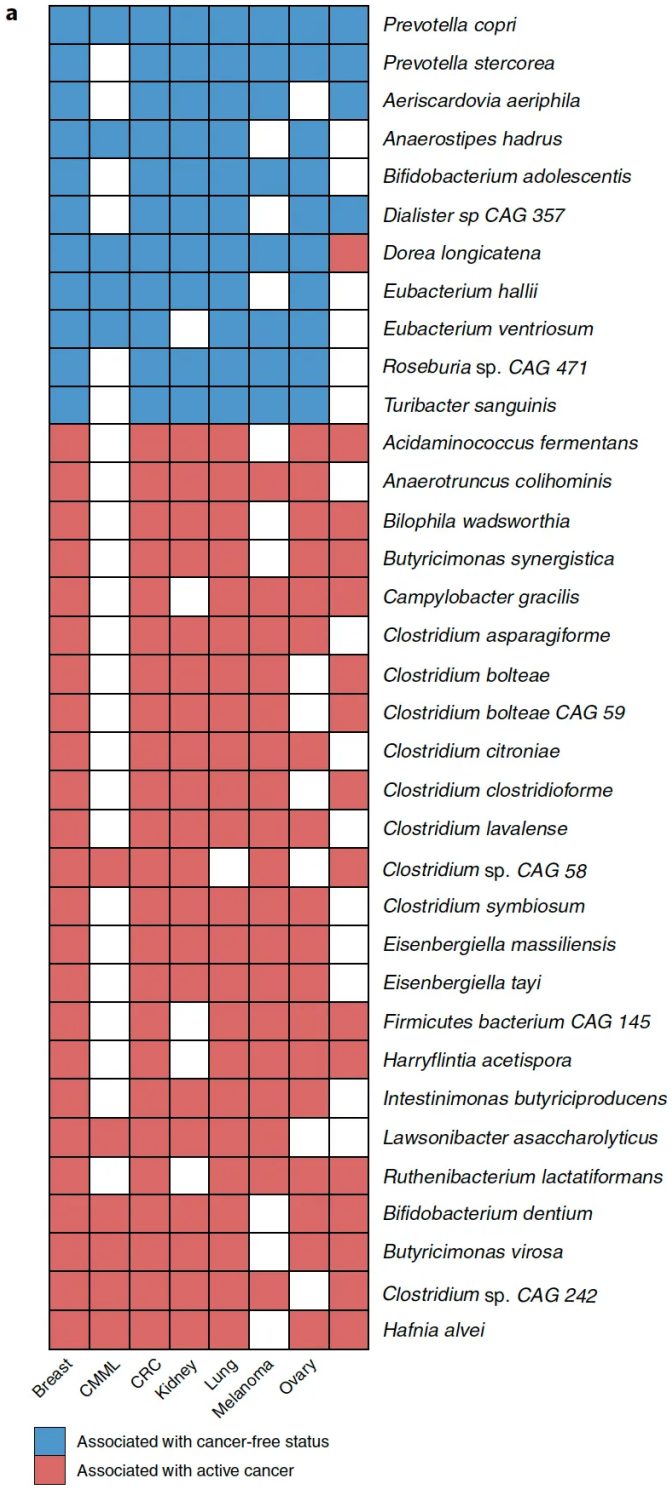

Figure 8.1: Microbes by cancer type

Jeff Lapides and others from Drexel find Evidence supportive of a bacterial component

> We detected bacteria in the brains of both cohorts with the principal bacteria comprising Cutibacterium acnes (formerly Propionibacterium acnes) and two species each of Acinetobacter and Comamonas genera.
>
> The AD-related pathogenicity of the brain microbiome seems to be based on a complex polymicrobial dynamic. The time ordering revealed a rise and fall of the abundance of C. acnes with pathogenicity occurring for an off-peak abundance level in association with at least one other bacterium from a set of genera that included Methylobacterium, Bacillus, Caulobacter, Delftia, and Variovorax. C. acnes may also be involved with outcompeting the Comamonas species, which were strongly associated with non-demented brain microbiota, whose early destruction could be the first stage of disease. Our results are also consistent with a leaky blood–brain barrier or lymphatic network that allows bacteria, viruses, fungi, or other pathogens to enter the brain.

8.5 Parkinsons Disease

See Jun 2022 Review: Gut Microbiota: A Novel Therapeutic Target for Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder that is the second-most common after Alzheimer's disease. Here is a general overview of PD and its known demographic factors:

1. Age of Detection:
    - Most people diagnosed with PD are age 60 years or older[10].
    - However, an estimated 5 to 10 percent of people with PD are diagnosed before the age of 50[11].
    - The overall incidence of PD increases with age[12].
    - The incidence of PD in persons ages 65 and older ranges from 108 to 212 per 100,000[13].
2. Sex:
    - Men are 1.5 times more likely to have Parkinson's disease than women.
    - The male-to-female ratio of PD may change with age, suggesting that the etiology of PD may vary across different age groups[14].

[10] https://www.ninds.nih.gov/current-research/focus-disorders/focus-parkinsons-disease-research/parkinsons-disease-challenges-progress-and-promise
[11] https://www.parkinson.org/understanding-parkinsons/statistics
[12] https://academic.oup.com/aje/article/157/11/1015/151509
[13] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2865395/
[14] https://jnnp.bmj.com/content/87/9/952

3. Geography:
    - The prevalence and incidence of PD can vary by geographic location.
    - A study in North America found that the age-sex-adjusted incidence of PD ranged from 108 to 212 per 100,000 among persons ages 65 and older[15].
    - Geographic and ethnic variations in PD have been observed, but further research is needed to understand the underlying factors.

It's important to note that these demographic factors are based on available research and may not capture the full complexity of PD. The understanding of PD is constantly evolving, and further research is needed to uncover additional factors that may contribute to the development and progression of the disease.

Later I'll describe my own Personal Science observations about the link with Parkinsons

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Also see the link with trichloroethylene (TCE), used to decaffeinate coffee, degrease metal, and dry clean clothes

8.6 Other conditions

Some people claim you can treat migraine headaches, sinusitus, and other conditions by inserting *Lactobacillus*-containing Kimchi up the nostril. I've not tried it and can't vouch for it, but let me know if it works for you.

8.7 Microbes and Behavior

Your gut contains 100 million neurons[16], which incidentally is about the same as an entire mouse. With all those neurons touching microbes, it's not surprising that there are links with behavior.

Toxoplasma Gondii is a tiny microbe that, for some reason, only likes to reproduce from within the gut of a cat. It can be found in almost all warm-blooded mammals, including humans — about 30% of us, according to some estimates, and that's after a century of obsession with hygiene that has wiped out countless other tiny inhabitants of the body.

T. Gondii seem harmless because its hosts appear to show no differences before or after "infection" except in one creature: the rat. In this classic experiment[17], rodents that were infected

[15]https://www.nature.com/articles/s41531-022-00410-y
[16]Courage (2019) p.46
[17]Vyas et al. (2007)

with *Toxoplasma gondii* were studied with special dyes that can show how the infection spreads, first in the gut and then to the brain.

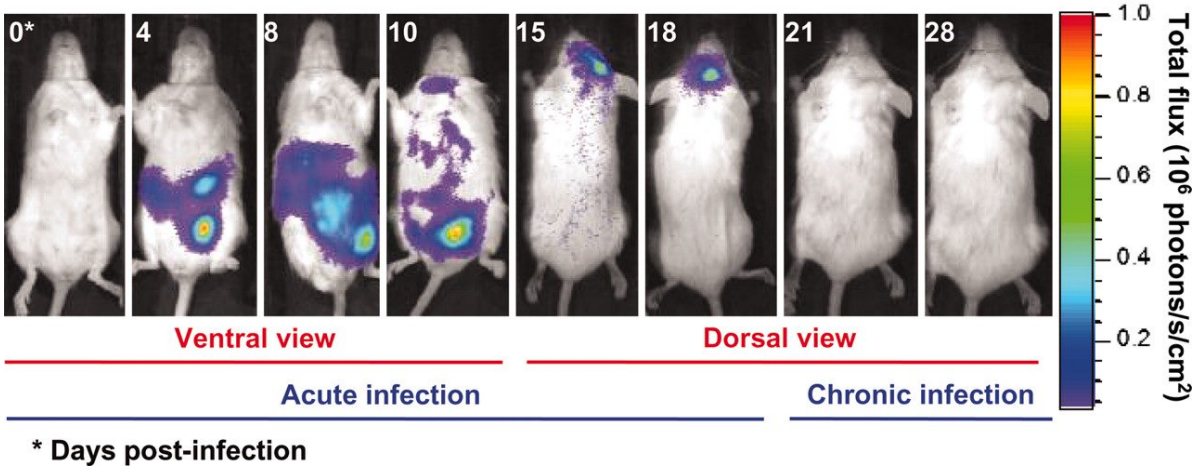


Figure 8.2: Twenty-eight days after infection, the rat brain is permanently altered

When it finds itself ingested by a mouse or rat, it appears to fade away quietly. MRI scans show large amounts in the gut for a week or two, gradually decreasing until there is apparently nothing. By day 18, the last remnants of the infection are disappearing from the brain, except for tiny T. Gondii cysts. Not everywhere, but in just a few strategic places.

Then something strange happens: the rat is now unnaturally attracted to cat urine. And note: its only attraction is to cat urine, not rabbits, not humans — only cats. Everything else about the rat appears normal. It still seems to be afraid of the other things that scare rats: other predators, stressful situations. But put a *T. Gondii*-affected rodent into a maze with different animal urine in the corners and it will rush to the cat side every time.

How is it that *T. Gondii* is able to be so precise in its effects? It seems to affect just rodents[18] , and even then it only apparently makes them attracted to cats. It doesn't apparently cause any other harm to its hosts, so — somehow — it must have found a resting place right at the spot in the mouse brain that affects its interest in cats.

Although there doesn't seem to be any major negative consequences to humans hosting *T. Gondii*, some scientists aren't sure. One man in particular, Jaroslav Flegr, an evolutionary biologist at Charles University in Prague, thinks he has evidence that women who carry it might be more trusting than those who don't.

Incidentally, some of the drugs used to treat schizophrenia have been shown to reduce levels of *T. Gondii*.

[18] new evidence shows it may make wolves more aggressive: https://www.nature.com/articles/s42003-022-04122-0

And *T. Gondii's* ability to cross the blood-brain barrier may make it a potential vector to deliver therapeutics, eventually helping to treat (or cure) brain illnesses like Alzheimers. A University of Glascow - Tel Aviv University team did just that with an engineered version that successfully delivered the MeCP2 protein to the correct target location in brain organoids. [19]

T.Gondii isn't the only microbe known to affect the behavior of its host. A more common example is the rabies virus, which upon infection somehow causes a mammal to be more agitated, more likely to strike out — or bite — other humans, thereby spreading itself.

Or syphilis, the disease spread by *Treponema pallidum* that causes its host to go insane. The microbe is somehow able to infect the mind of the victim.

In general, sexually transmitted diseases are especially likely to have behavioral consequences. A sexual disease that produces symptoms is unlikely to spread, yet it still requires contact with a new victim. Perhaps the ideal vector is a behavioral change, making the host more likely to come in contact with a new host.

A well-done 2019 study[20] found that people suffering from depression have significantly lower levels of two groups of bacteria, *Dialister* and *Coprococcus*, possibly due to a potential ability of the gut microbiome to synthesize 3,4-dihydroxyphenylacetic acid, a breakdown product of the neurotransmitter dopamine.

There are many other examples of microbes that appear to affect the brain:

- 5-HTP is an intermediate molecule between tryptophan and serotonin. It is produced by *Candida*, *Streptococcus*, *Escherichia*, and *Enterococcus*.
- *Bacillus* and *Serratia* make dopamine
- *Escherichia*, *Bacillus*, *Saccharomyces* make noradrenaline
- *Lactobacillus* can produce acetylcholine
- GABA can be produced by *Lactobacillus* and I.

Bacteroides Fragilis is depleted in autistic patients. it is a gatekeeper for the immune system [http://www.ncbi.nlm.nih.gov/pubmed/16009137]

Sutterella may have implications for autism, causes tics ?

[19] Bracha, S., Johnson, H.J., Pranckevicius, N.A. et al. Engineering Toxoplasma gondii secretion systems for intracellular delivery of multiple large therapeutic proteins to neurons. Nat Microbiol (2024). https://doi.org/10.1038/s41564-024-01750-6

[20] Valles-Colomer et al. (2019)

Bifidobacterium infantis: see Sudo, Chida for gnobiotic mice that it prevents from becoming stressed.

Mark Lyte and his colleagues, a microbiology team from the Minneapolis Medical Research Foundation, studied the effect of infecting mice with *Campylobacter*, one of the bacteria implicated in the Walkerton epidemic. The dose of bacteria was high enough to be detected in the intestine, but not so high that the mice developed overt illness. You probably won't be surprised to learn that the campylobacter-infected mice exhibited more anxiety when navigating a maze than the control mice.

Lactobacillus rhamnosus is lower in pups born to pregnant mothers under stress. Intrigingly, this species is linked to levels of GABA, an important neurotransmitter targeted by anti-anxiety drugs like Valium and Xanax.[21] What's more, in mice the action of these microbes seems modulated by the vagus nerve – mice who have their vagus nerve removed seem to be unaffected by GABA.[22]

Scientists in Japan characterized the microbiome of 25 anorexia nervosa patients and compared them to healthy controls. The AN patients had a lower amount of total bacteria and specifically, lower amounts of *Clostridium coccoides* group, *C. leptum* subgroup, *Bacteroides fragilis*, and *Streptococcus*.[23]

Or consider the bacterium *Tropheryma whipplei*, the infectious cause of Whipple's disease. Sufferers often have neurological symptoms like memory loss and odd eye and face movements called *oculomasticatory myorhythmia*, which indicate that somehow the microbe has invaded the nervous system.

Autism (which we'll discuss later), is often accompanied by a strange craving for propianate-heavy products like bread. You can see videos showing how rats behave when given too much propionate.

We all know people who seem exceptionally fastidious, some who are diagnosed with Obsessive Compulsive Disorder (OCD). The bacterium *Streptococcus* become relatively more abundant after hand-washing, so they influence the basal ganglia to do more hand-washing? Is that a coincidence?

Think about this too much, and you'll end up with the obvious question: what other weird microbes are infecting us right now? Can we explain some of our own behaviors this way? Is there a human equivalent of these infections, driving us to do things we "ordinarily" wouldn't do? And maybe these microbes are so ubiquitous, teeming all over us and in our brains, maybe there's no way to even know what "ordinary" or "normal" human behavior is.

[21] See a detailed discussion in the New York Times: https://www.nytimes.com/2015/06/28/magazine/can-the-bacteria-in-your-gut-explain-your-mood.html

[22] Bravo et al. (2011)

[23] http://www.microbiomeinstitute.org/blog/2016/1/7/gut-dysbiosis-in-anorexia-nervosa-patients

Part III

Technology

9 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.

9.1 The technology for studying microbes

People have been farming the microbes in fermented foods for thousands of years, so when in Pasteur times, scientists first began to cultivate them for experiments, the most obvious way was through the process known as "culturing". Take a sample containing some microbes of interest, and leave them sit in a hospitable environment long enough for them to reproduce in enough quantity to be useful. That's still a common way to study microbes, and that couple-of-day incubation period is one reason you don't get your lab tests back for a few days.

Culturing also has several serious limitations. It only works if the microbes are still living, which rules out many important situations. Many microbes don't culture well or at all outside their native habitat.

Anaerobes are organisms that can't survive in the presence of oxygen, not a problem deep inside the airless gut, but it won't work in a normal lab. While you can take some precautions to preserve the original environment as much as possible – you can set the organisms in a specially-sealed oxygen-free container –the cost and expense rises quickly.

Even if, somehow, you were able to overcome all the other challenges, many (perhaps most) microbes don't grow well unless they are in close proximity to other specific species. *Methanobrevibacter smithii*, for example, which plays a critical role in the efficient digestion of complex sugars, removes hydrogen from its environment, providing a habitat for organisms that don't like hydrogen, like *Firmicutes* and *Bacterodetes.* Plus, it converts all that excess hydrogen to methane, which in turn is needed by yet other organisms. Culturing any of those microbes on their own would be difficult, if not impossible.

But the techniques for uncovering which organisms are where and what they are doing was revolutionized in the first decade of the 2000s by those new-fangled gene sequencers that were so usefully applied to human genes.

9.1.1 The 16S rRNA Gene

Despite the plunging costs of DNA sequencing, the trillions of microbes in your gut still present a formidable chellenge if you intend to sequence them all. Even the humble *E. coli* contains nearly five million DNA letters. There is some commonality between related organisms — humans and chimpanzees, for example, share upwards of 90% of their DNA — but in general it's hard to use the DNA strand itself to measure the relatedness between two organisms. Understanding the reason for this may help you understand why there is a clever shortcut.

You might think you can measure the relatedness of two organisms by looking at all the DNA in each one and computing the percentage that each shares in common. This would work, but sequencing all those billions of DNA bases takes a lot of time and money, and it would be impractical in a case like the microbiome where you may need to do this for millions of individual organisms.

A service like 23andme is able to cheaply compare individuals of the same species (i.e. Humans) because the generic human genome is already well-mapped and we know that of the 3 billion base pairs, only about 3 million (the SNPs or single-nucleotide polymorphisms) are different between individuals. When you give your spit sample to 23andme, they give you back a subset of your SNPs, only those that have been studied enough to be interesting. SNPs are easy and cheap to find using a "gene-chip", a special semiconductor-like device that can quickly look at 1 million or more pre-determined spots on your DNA. But this is only possible because the map itself already exists, thanks to multi-year effort of the Human Genome Project that finished in the early 2000s. There are no comprehensive gene chips (yet) for bacteria, and certainly not for all the millions of species in nature. And even if there were such chips, bacteria are notorious at adapting and changing to their surrounding environment, exchanging genes with one another, that it just wouldn't be practical to identify enough constant genes to make it worthwhile.

Fortunately, to get an overall picture of the types of microbes in your body, we don't have to sequence every piece of DNA. For our purposes, we just want to know *which* organisms are there, and in what abundance. The precise bits of DNA are important only if they let us know the names of the microbes, and for this we don't need to bother sequencing everything. In fact, most bacterial species differ enough from each other that we need only a few bits of DNA from each in order to tell them apart.

We know that all bacteria are distantly related to one another, and that closely-related species will have more DNA in common with each other. But some of parts of DNA are so important that they stay virtually identical even across entire families of organisms. Remember that DNA describes absolutely *everything* about the organism, including the workings of very low-level cell process. Not just the size or shape, but much more fundamental: how a cell divides, for example, or even how to use the oxygen a cell needs for survival.

Among the most fundamental of all processes is what happens in every cell's ribosome, a special molecule that is core to how a cell converts DNA into proteins. Because all cells create

proteins, they also always contain a ribosome and, importantly, they contain the *instructions for how to create a ribosome* in the form of the ribosomal gene. Each cell's DNA includes a gene that precisely encodes every protein, in the exact order that makes up the ribosomal structure. A special enzyme, called DNA polymerase, manufactures new bits of RNA on the fly as it hits portions of the DNA. These bits of RNA, called messenger RNA or mRNA, eventually make their way to ribosomes, which are floating throughout the cell. Upon hitting the ribosome, mRNA is converted into the proteins that make all life possible. If it happens that the mRNA hits upon a segment of DNA that encodes a gene for a ribosome, guess what new molecule is manufactured? A new ribosome!

This ribosomal gene is such a fundamental part of every living organism that very little about the ribosome changes, even after hundreds of millions of years of evolution. Humans and corn plants actually share quite a bit of the ribosome; both are prokaryotes, for one thing, so many of our cellular processes work the same. But bacteria go back even further than humans and corn plants, enough so that the differences aren't so subtle anymore. In fact, the differences are big enough that, with clever selection of the part of the genome to sequence, you can tell the difference between two bacteria in a few hours for a fraction of the cost of running through all the DNA you might find in a microbiome.

The gene that encodes ribosomal RNA (written rRNA) for bacteria consists of about 1500 base pairs total, a tiny fraction of the entire genome, and although it is mostly identical across all bacteria, there are *some* differences, all of which are contained in nine "hypervariable" regions containing even fewer base pairs. These regions, named V1 through V9, are surrounded by strings of base pairs that are constant throughout all bacteria, and can be quickly discovered and amplified by the right DNA primers. The fourth one of these regions, V4, contains only 250 base pairs, and is quickly and easily sequenced on commercially-available sequencing machines.

When you submit your sample to a lab, the bacterial cells must first be cut into pieces ("lysed", to use the technical term). Sometimes the first part of this process happens at collection time, when you swab a tiny bit of your sample into a vial and stir. The vial contains tiny "beads" that smash into the cell walls as you stir, breaking them apart to spill their contents in an ugly liquid "goo".

The lab is interested only in the DNA inside that goo, so they start by dropping in some carefully-constructed "primers". These are bits of known, synthetically-made DNA that are designed to bind just to the parts of the cell DNA that make ribosomes. In particular, these primers will only find bits of DNA that make the specific, V4 subregion of the ribosome. Primers naturally bind and then break open the DNA at precise locations, cutting out all the segments that match.

Throw this goo into a centrifuge spinning at a carefully controlled, very high speed, and different parts of the goo will rise to different levels, reflecting their molecular weights. One specific part, corresponding to the section programmed to make ribosomes, will rise to a centrifuge level referred to as "16S". Precisely skimming the goo at that spot will give the

technician a collection of DNA from just one part of the ribosome of bacteria. The rest of the DNA, millions of letters (base pairs) per bacteria, will not be sequenced and is simply discarded. That's the shortcut. Instead of sequencing millions of base pairs, we need sequence only hundreds.

Once you have a bunch of that 16S ribosomal gene, you know that you are looking exclusively at non-human bacterial and archaeal DNA. It's a tiny subset of all the genetic information in the microbiome, but combined with one more shortcut, it gives a surprisingly accurate look at the overall composition of a sample.

The remaining shortcut is possible thanks to years of research of sequencing the genes in bacteria. Scientists in labs around the world have been faithfully digging up samples of bacteria, and performing whole-gene sequencing on what they find. Although 250 base pairs may seem like a tiny number to differentiate among all possible bacteria on earth, for gut microbiome purposes we need concern ourselves only with those that are known to inhabit humans. The Human Microbiome Project already identified most of these bacteria – and their 16S gene identifiers – so armed with that as a reference database[1], it is generally possible to unmask a specific microbe with just a sliver of DNA.

It's this two-step combination, 16S "skimming" and a database lookup, that makes it cost effective to study the millions of organisms in your microbiome. You don't have to do a complete gene sequence on every single bacterium; just trust that the tiny subset of DNA in the 16S region is enough to uniquely match something already in the bacterial database.

The alternative – sequence everything in the sample – provides much more accuracy of course, but the 16S approach comes surprisingly close. Careful studies that compare with the "sequence everything" (aka metagenomic) approach show that 16S is still surprisingly close – at least 80% and often much more of the entire microbiome can be categorized accurately, even at the species level.

9.1.1.1 Limitations of 16S

While 80%+ accuracy for such a cheap and fast method of sequencing is impressive, it's important to remember that we're still not seeing the whole story. Despite its low cost and wide use, microbiome studies that focus only on the 16S gene suffer from several inaccuracies compared to other, more expensive methods.

For one thing, this type of sequencing sees only the bacteria in a sample. Other important single-cell, invisible microbes won't be detected: yeasts, fungi, and most archaea. Viruses, including phages that prey on bacteria, are also not part of the 16S summary.

The thousands of genes in each of the trillion bacteria in your system are doing important work that won't be visible if we sequence a portion of just one of them. Much of the time,

[1] A very popular one is Greengenes: http://greengenes.secondgenome.com/downloads. Learn about all the big ones (Balvočiūtė and Huson 2017 Fig 3)

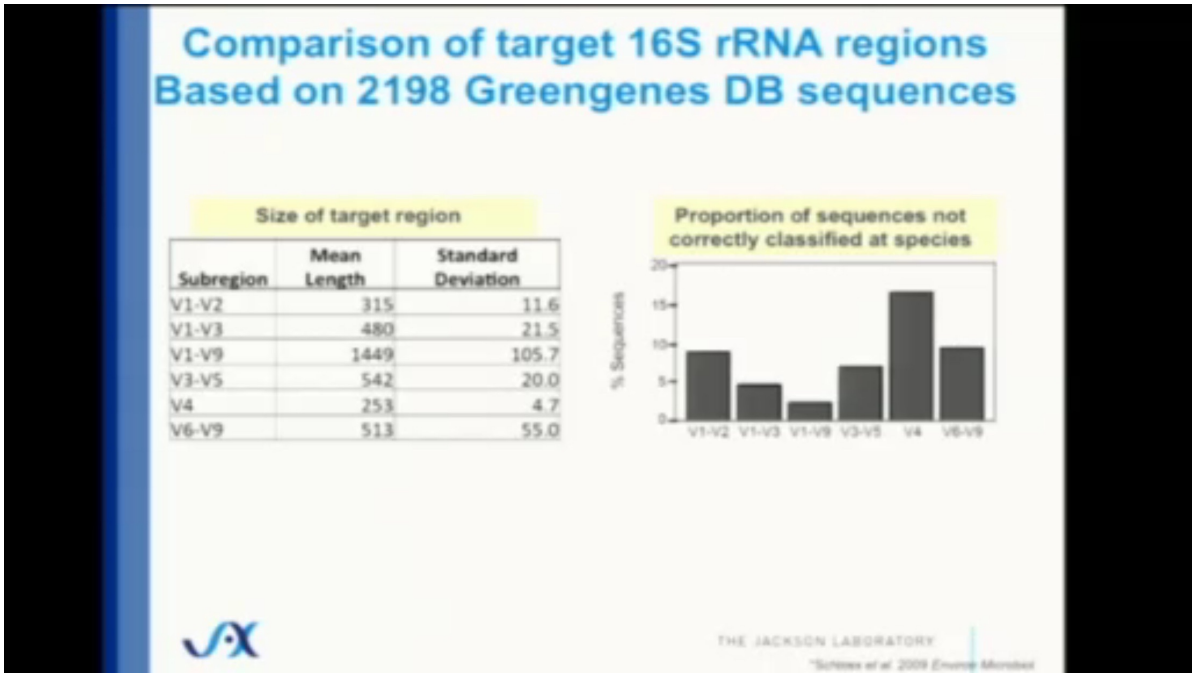


Figure 9.1: Just 253 base pairs of the V4 subregion are enough to correctly classify more than 80% of the species in a sample. (Source: Jackson Laboratory)

this doesn't matter, because the vast majority of the unsequenced portion is genes that are identical to those that have already been sequenced by previous scientists. To use an analogy in the visible world, if you have identified that an organism is a bird, it's very likely – though not certain – that it can fly. For nearly all bird species that would be a safe bet, but among the birds *that actually matter to humans*, you'd have an important detail wrong about chickens.

Other problems with 16S microbiome testing technology: it's limited in what it can see, and RNA itself is too unstable. One careful study concluded:

> "16s rRNA predicts genome-wide levels of similarity very well for distantly related prokaryotes, but not for closely related ones"[2]

Worse, there are many important bacteria that share identical 16S sequences yet occupy entirely different ecological niches [3]. Because the sequences are identical, different labs may arbitrarily assign different names to the same organism. [4]

[2]Lan, Y., Rosen, G., & Hershberg, R. (2016). Marker genes that are less conserved in their sequences are useful for predicting genome-wide similarity levels between closely related prokaryotic strains. Microbiome, 4(1). https://doi.org/10.1186/s40168-016-0162-5

[3]Jaspers and Overmann (2004)

[4]a good summary is in Pollock et al. (2018)

9.1.2 Limitations of collection

Another source of possible error depends on how the sample was collected, and how it was handled after collection. The gold standard of collection requires a subject to be physically present in a lab, where the sample is collected and sequenced immediately, or else quickly frozen and then pulverized into tiny pieces that are carefully blended and then sequenced. Not only is that expensive, but it requires the subject to "poop on demand", which isn't always feasible. A common alternative asks the subject to place all or a scoop of the sample into a freezer which is sent to the lab later.

Most home-based collection methods require you to collect a tiny swab of material which is then placed in a vial for shipment through the mail. The vial usually contains a special buffering chemical that keeps any DNA inert during transit. Because DNA is generally pretty stable, a vial preserved this way can usually remain usable for months or even years at room temperature.

How much does that affect the final result?

Several studies have tried to compare collection methods, with mixed results. The most systematic study, performed by scientists at the Centers for Disease Control tested 8 hospital patients.[5] Collecting samples from the bowel movements as well as rectal swabs inserted up the you-know-where at specific time periods, the researchers concluded that the differences *within* an individual are much smaller than the differences *between* individuals. In other words, although a single sample may have some variability depending on exactly where you swab, it won't matter if you're comparing to somebody else.

I tried several different ways of collecting samples, and discovered that the results do indeed depend greatly on the sampling conditions. See my detailed results in the Experiments: collection chapter.

The above limitations are important, and there's no question that you should keep them in mind when exploring your own microbiome, but the low price and accessibility of the technology makes up for it in many important applications.

Thans to these new machines originally developed for mass DNA sequencing, the process of finding and understanding microbes has been revolutionized. It's now possible to search for new life forms *without* growing them in a culture, and this has made possible a major shift in how to think about life —and what is important and special about human hardware.

Unlike the genetic discoveries you can make by understanding your DNA (from a low-cost consumer service like 23andme), much of the news from the microbial world is *actionable*.

[5] Bassis et al. (2017)

There's little, if anything, you can do if you find you have a particular type of gene that gives you, say, a propensity to alzheimers for example. But because the microbes around you are constantly changing anyway, and because you can influence which ones grow and which don't, the world of the human micro biome is eminently *actionable*.

10 Handling Microbiome Data

A thought experiment about microbiome testing:

Imagine standing outside a stadium you're told is full of marbles, with trucks driving in and out all day. Monday morning you catch one out-going truck and count 100,000 marbles, of which 30,000 are red. A week later, you find another truck leaving with 200,000 marbles, of which 20,000 are red.

Question: What can you say about abundance of red marbles?

a) The total number of red marbles in the stadium has gone down. The percentage dropped from 30% (30k/100k) the first week to only 10% (20k/200k) the next week.

b) The total of red marbles stayed the same, but somebody added a bunch of differently-colored marbles that diluted the share of red.

c) You can't say much with certainty one way or another, even if you can assume each truck holds a representative sample of the stadium. You have to track the *ratio* of colors in each truck. Simply knowing the percentage of one color is meaningless.

I asked this question on the Facebook Gut Club

10.1 A word about microbiome sequencing

When a microbiome sample is sequenced by a genetic sequencing machine, the results are presented in large files, called FASTQ, made of the A, C, T, G letters of the genetic code along with other information about measurement accuracy and more. The final report sent to you as a customer, builds from these files using a bioinformatics "pipeline" designed to summarize the genetic code into a more readable format. Embedded within the pipeline are dozens of assumptions about how to best interpret the genetic letters, including how to handle cases where the interpretation is unclear, or even arbitrary. For example, although the sequence of a common microbe like *Streptococcus mutans* is well-understood, how close does a sequence have to be before the report can confidently describe it as a member of that species? Different pipelines make different assumptions. One might say it can be off by 10 letters, while another might say 5; other pipelines might judge based on the particular microbe. And what should the report do when the sequencer returns less-than-confident results? No sequencer can be

perfect all of the time, so by necessity some allowance must be made for how much leeway should be allowed in an interpretation.

10.2 Microbiome datasets are compositional

Once the pipeline has been tweaked to give consistent answers for a particular lab, another question awaits.

Since most tests report the *relative* abundance of a particular microbe, the totals will always sum to 100%. While this makes sense when you want to know the overall composition of the microbiome, it may not be as useful when studying how the results from one day compares to another.

The reason is *compositionality*, sometimes called the "sum to 1" problem. To explain this, let's use a concrete example.

10.3 Example

Suppose we have the following result for our first test:

Test 1		
Microbe	Absolute	Relative
A	100	10%
B	500	50%
C	400	40%
D	0	0%
Total	1000	100%

We don't specify the units in the "Absolute" column, but it can be whatever you like: grams, tons, mg/mL – it doesn't matter. In this simple example, we measure a total of 1000 (of something) and compute the various relative amounts. All is well.

In our second test, for whatever reason, we collect a lot more stuff, leading to a larger *absolute* amount but the relative amounts are unchanged.

Test 2		
Microbe	Absolute	Relative
A	150	10%
B	750	50%

Test 2		
C	600	40%
D	0	0%
Total	1500	100%

But now consider a different case. This time, for some reason one of the three microbes has a massive increase in absolute terms. Importantly, *none of the other microbes changed*. This might happen if your sample were somehow contaminated, for example, perhaps from some extraneous microbe entering the tube after you sampled it. Or it could be that the sampling site suddenly had a new growth of an new microbe that doesn't affect anything else. Lots of reasons could explain why the *absolute* values of various microbes could be unchanged even the *relative* values are substantially different.

Test 3A		
Microbe	Absolute	Relative
A	150	8%
B	750	38%
C	600	30%
D	500	25%
Total	2000	100%

But you don't need contamination for a slight change in one microbe to have a major impact on the *relative* abundance of the others.

Watch what happens when two microbes, A and B, are unchanged while two others swap abundance amounts.

Test 3B		
Microbe	Absolute	Relative
A	150	8%
B	750	38%
C	700	35%
D	400	20%
Total	2000	100%

A and B appear to have the same relative abundances they did in Test 2. This simple case matches our intuition: we expect that the relative values of A and B would be no different than Test 3A. The absolute totals are the same, so again all is well.

But microbes exist in an *ecology*. They're not independent of one another. Often an increase or decrease in one will drive a corresponding change in another.

Consider the interesting case where one one microbe (A) doubles in abundance, causing another (B) to halve. Although the changes are directly related to one another, it's hard to see that in the type of relative summary we get from our report.

Test 2B		
Microbe	Absolute	Relative
A	200	24%
B	250	29%
C	400	47%
D	0	0%
Total	850	100%

In 2B, a major change happened – the abundance of one microbe (A) exploded and caused another (B) to plunge. Although another, independent microbe (C) was completely unaffected by this change, when we look only at the relative differences, we might be fooled into thinking that C changed as well, though it didn't.

Which matters more, absolute values or relative ones? To the extent that the microbiome is synthesizing or digesting various metabolites in the body, it's clear that *absolute* values are what we want to watch. But absolute abundances are too hard to track – you'd need to grab the entire microbiome somehow. So instead we assume that the microbiome *as a whole* maintains a roughly constant absolute volume and that the only change is the relative abundances.

Is that true? It seems unlikely. Other living populations rise and fall depending on all sorts of factors. Your backyard garden, for example, doesn't have the same absolute volume from one day to another. If you only knew the *relative* percentage of tomatoes versus cucumbers, would you really know much about your harvest?

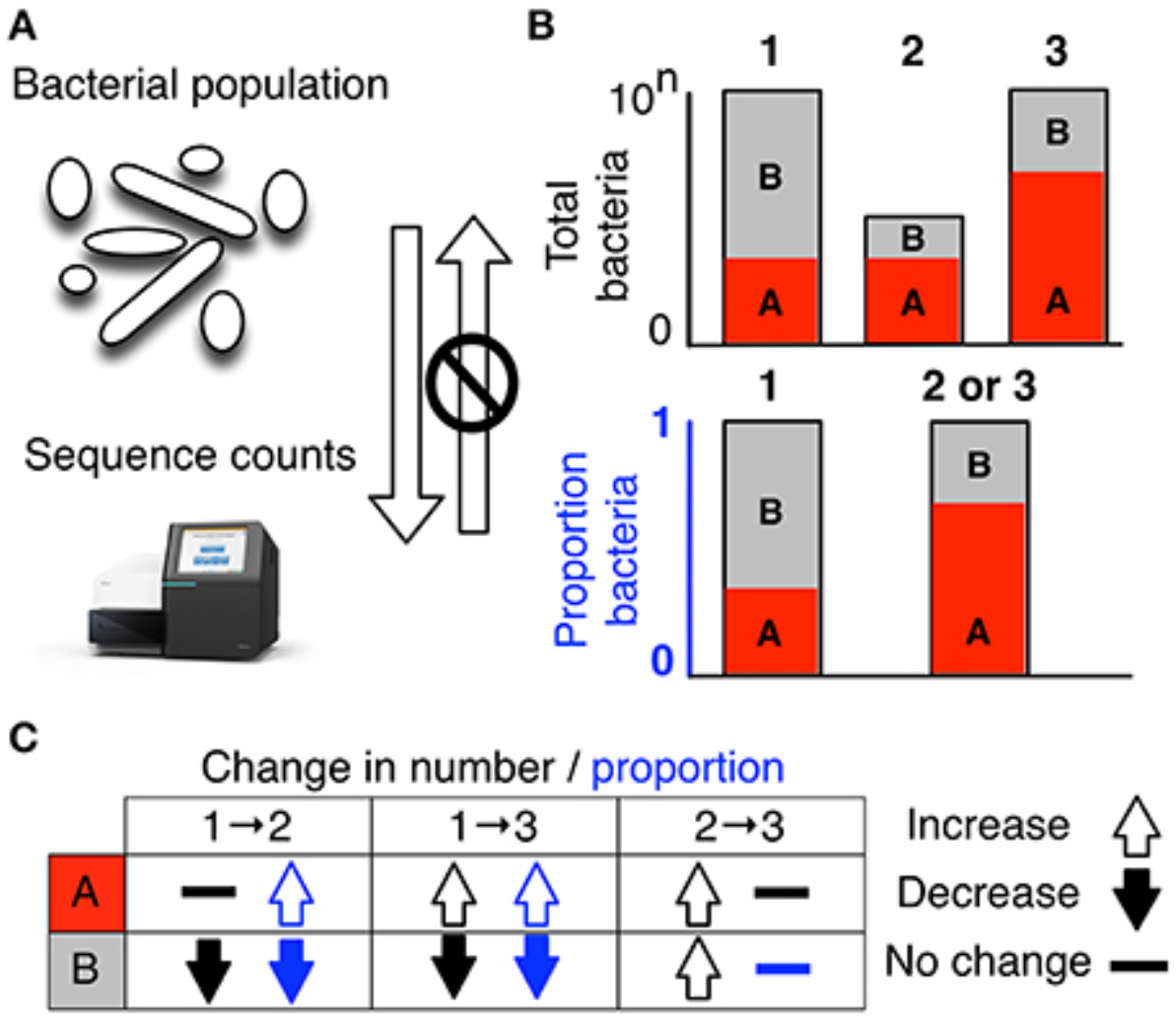


Figure 10.1: FIGURE 1 (from Gloor et al. (2017))) High-throughput sequencing data are compositional. (A) illustrates that the data observed after sequencing a set of nucleic acids from a bacterial population cannot inform on the absolute abundance of molecules. The number of counts in a high throughput sequencing (HTS) dataset reflect the proportion of counts per feature (OTU, gene, etc.) per sample, multiplied by the sequencing depth. Therefore, only the relative abundances are available. The bar plots in (B) show the difference between the count of molecules and the proportion of molecules for two features, A (red) and B (gray) in three samples. The top bar graphs show the total counts for three samples, and the height of the color illustrates the total count of the feature. When the three samples are sequenced we lose the absolute count information and only have relative abundances, proportions, or "normalized counts" as shown in the bottom bar graph. Note that features A and B in samples 2 and 3 appear with the same relative abundances, even though the counts in the environment are different. The table below in (C) shows real and perceived changes for each sample if we transition from one sample to another.

10.4 The solution

This problem has been noticed for more than a hundred years in every field touched by statistics: ecology, economics, geology and more. Whenever you have an instrument that can only measure a subset of something, you must make allowances for the fact that the final measure is reported in units of 100%.

The solution is to make calculations based not on overall *percentages*, but on ratios of each component. The statistics are more complicated, but that's the only way to make the final result usable.

10.5 Bottom line

It's very hard to make judgements one way or another from simple comparisons of relative abundance changes from one sample to another. Too many factors determine the measured levels of the various microbes.

Despite this, we know empirically that the overall relative abundances are reasonably stable from one collection to another. Not precisely stable, but at least at the highest, say, phylum levels, the abundances track fairly consistently from day to day. In the oral microbiome, for example, *Streptococcus* is almost always the lead phylum, with *Neissaria* and *Rothia* competing with a few others for second or third place.

Meanwhile, in larger population studies of say thousands of people sampled multiple times, some significant patterns emerge of microbes that are consistently over- or under-represented in various disease states.

Or consider our garden analogy. Knowing the relative percentage of tomatoes and cucumbers might be useful if we had data meticulously collected from thousands of other backyard gardens, along with some "metadata" about each gardener's assessment of their harvest. You might notice, then, that gardeners unhappy with their tomato crop tend to have lower cucumber yield too. Or there might be a strong correlation between tomato yield and herbicide usage – on average. Still, many or perhaps even most gardens will be significantly different. For example, if the relative abundances appear to match the average, you might be fooled into thinking that a garden suffering from an overall poor harvest is fine.

In other words, treat numbers like "relative abundance" with an appropriate level of skepticism.

Gloor et al. (2017)

Part IV

Do It Yourself

11 Microbes to Watch

Your gut as seen by consumer-priced sequencing technology contains many more unique microbial species than you can possibly track, at least hundreds in most people and potentially over 1000. I've seen 1083 different ones in my own results. And that's just using the comparatively crude 16S technology. More comprehensive estimates based on other technology find as many as 36,000 different species[1]! With that much variety, how do we find the ones that matter?

Fortunately, only about 14 strains of 10 species account for 80% of a typical gut microbiome[2]

In this chapter, 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 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 overall abundances of some key microbes.

11.1 Phylum

> **!** Important
>
> This section is under construction

In biology, a phylum (/ fa ləm/; plural: phyla) is a taxonomic rank used to classify organisms. It is a group of related classes. The term was coined by Ernst Haeckel in 1866.

Traditionally, in botany the term division has been used instead of phylum, although the International Code of Nomenclature for algae, fungi, and plants accepts the terms as equivalent. Depending on definitions, the animal kingdom Animalia contains about 31 phyla, the plant kingdom Plantae contains about 14 phyla, and the fungus kingdom Fungi contains about 8 phyla.

[1] See (Frank et al. 2007) or click for the open access download
[2] See the detailed estimates here: (Kraal et al. 2014)

At its most basic, a phylum can be defined in two ways: as a group of organisms with a certain degree of morphological or developmental similarity (the phenetic definition), or as a group of organisms with a certain degree of evolutionary relatedness (the phylogenetic definition). Attempting to define a level of the Linnean hierarchy without referring to (evolutionary) relatedness is unsatisfactory, but a phenetic definition is useful when addressing questions of a morphological nature—such as how successful different body plans were.

The concept of phylum is based on the idea that organisms that share a common ancestor are more closely related to each other than organisms that do not share a common ancestor. This means that organisms in the same phylum are more likely to have similar characteristics than organisms in different phyla.

For example, all the animals in the phylum Chordata share a common ancestor that had a notochord, a rod-shaped structure that supports the body. This means that all chordates have a notochord at some point in their development.

The concept of phylum is also based on the idea that organisms in the same phylum are more likely to have a similar evolutionary history than organisms in different phyla. This means that organisms in the same phylum are more likely to have evolved from a common ancestor in a similar way.

For example, all the animals in the phylum Chordata have a common ancestor that lived about 500 million years ago. This ancestor was a small, worm-like creature that lived in the ocean. Over time, this ancestor evolved into the different types of animals that we see today, including humans, fish, and birds.

The concept of phylum is a useful tool for classifying organisms and understanding their evolutionary relationships. It is also a useful tool for studying the diversity of life on Earth.

The gut microbiome of most westerners is dominated by *Firmicutes* and *Bacteroidetes*, which together make up 80% or more of the total sample. Most people also have smaller amounts of *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia*. This overall composition is so common in healthy people that it's tempting to assume their dominance 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.

11.2 Genus

> **!** Important
>
> This section is under construction

In biology, a genus is a taxonomic rank used to classify organisms. It is a group of species that are closely related to each other. The genus name is always capitalized and comes first in the binomial nomenclature of a species. For example, the genus name for humans is Homo, and the species name is sapiens.

The concept of genus was first introduced by the Swedish naturalist Carl Linnaeus in his 1753 work Species Plantarum. Linnaeus divided all living things into three kingdoms: plants, animals, and minerals. He then divided each kingdom into classes, orders, genera, and species.

The genus is a useful tool for classifying organisms because it allows us to group together species that share similar characteristics. For example, all the species in the genus Homo share the following characteristics: they are bipedal, they have large brains, and they use tools.

The genus is also a useful tool for understanding the evolutionary relationships between organisms. Species that are closely related to each other are usually placed in the same genus. For example, humans and chimpanzees are both placed in the genus Homo. This suggests that humans and chimpanzees are closely related, and that they share a common ancestor.

The genus is an important part of the biological classification system. It is a useful tool for grouping together organisms that share similar characteristics, and for understanding the evolutionary relationships between organisms.

In the context of the human microbiome, the genus is a useful way to group together different types of bacteria. For example, the genus *Lactobacillus* contains many different species of bacteria that are found in the human gut. These bacteria play an important role in digestion and immune function.

The genus is also a useful way to study the evolution of the human microbiome. By comparing the genomes of different species of bacteria in the same genus, we can learn about how these bacteria have evolved over time. This information can help us to understand how the human microbiome has changed in response to changes in our environment.

The term "genus" may not make intuitive sense to somebody used to thinking of eukaryotes or other organisms that reproduce via gametes. This is because the concept of genus is based on the idea of shared characteristics, which is not always clear-cut in the case of prokaryotes.

For example, the genus *Escherichia* contains many different species of bacteria that are very different from each other in terms of their appearance and their metabolism. However, they all share a common ancestor and they all have a similar DNA sequence. This is why they are all placed in the same genus.

Another example is the genus *Lactobacillus*. This genus contains many different species of bacteria that are found in the human gut. They all have a similar appearance and they all ferment carbohydrates. However, they have different DNA sequences and they are not all closely related to each other. This is why some scientists believe that the genus Lactobacillus should be divided into several different genera.

The concept of genus is also complicated by the fact that prokaryotes can reproduce asexually. This means that they do not produce gametes, and they do not have a sexual cycle. As a result, it can be difficult to determine how closely related two species of prokaryotes are.

Despite these challenges, the concept of genus is still useful for classifying prokaryotes. It allows us to group together organisms that share similar characteristics, and it can help us to understand the evolutionary relationships between organisms.

You're likely to hear most about the genus level because it's the most detail that cheap sequencing technologies can get right – most of the time.

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 (See Hacking Sleep.

11.3 Species

> **!** Important
>
> This section is under construction

When you hear the term "species", you probably think of a specific kind of creature, like a dog or a cat. More generally, among the kinds of plants and animals we encounter in the visible world, the term "species" refers broadly to organisms that can mate with one another to produce offspring of the same kind. Cats and dogs are different species because they can't mate with each other.

But bacteria don't mate: they reproduce by dividing themselves in half. So how do we define a species? In fact, even terms like "parent" or "child" aren't quite appropriate if each new cell is an identical copy of the old one. For very broad categories, like phylum or even genus, the similarities among like cells is high enough that we feel comfortable grouping them together with a common name, but at what point do we reach the lowest, most *specific* level.

The answer is tricky for another reason, called *horizontal gene transfer*, a process by which sometimes (in fact, quite often), a microbe will absorb genes from nearby organisms, altering its genome and its corresponding functions, sometimes significantly. Once that happens, the resulting new microbe can itself divide indefinitely, producing more and more copies of itself with the new gene. Although the new microbes still mostly resemble their original ancestor, if the new gene makes a protein that affects your body somehow, it might as well be an entirely different species.

The term "species" may not make intuitive sense to somebody used to thinking of eukaryotes or other organisms that reproduce sexually. This is because the concept of species is based on the idea of interbreeding, which is not always possible in the case of prokaryotes.

For example, the bacterium Escherichia coli can reproduce both sexually and asexually. When E. coli reproduces sexually, it produces two new cells that are genetically identical to each other. However, when E. coli reproduces asexually, it produces new cells that are not genetically identical to each other. This means that it is possible for two strains of E. coli to be genetically very different from each other, even though they are both members of the same species.

Another example is the bacterium Lactobacillus. This bacterium can also reproduce both sexually and asexually. However, Lactobacillus does not produce gametes, and it does not have a sexual cycle. As a result, it is not possible to determine how closely related two strains of Lactobacillus are based on their DNA sequence.

The concept of species is also complicated by the fact that prokaryotes can evolve very rapidly. This is because prokaryotes have a very simple genome, and they can replicate their DNA very quickly. As a result, it is possible for two strains of prokaryotes to evolve into two different species in a very short period of time.

Despite these challenges, the concept of species is still useful for classifying prokaryotes. It allows us to group together organisms that share similar characteristics, and it can help us to understand the evolutionary relationships between organisms.

Another way that "species" is different from our everyday usage of the term relates to the way microbial organisms are further differentiated by "strain".

A strain is a group of organisms within a species that share certain characteristics. Strains can be defined based on their physical appearance, their genetic makeup, or their response to certain environmental conditions. Strains are often used in microbiology to study the diversity of a particular species.

For example, there are many different strains of E. coli. Some strains of E. coli are harmless, while others can cause food poisoning. The strains of E. coli that cause food poisoning are typically more resistant to antibiotics than the harmless strains.

Strains can also be used to study the evolution of a particular species. By comparing the genomes of different strains of a species, scientists can learn about how the species has evolved over time. This information can help scientists to understand how the species is likely to respond to changes in the environment.

In other words, a strain is a group of organisms within a species that share certain characteristics. Strains can be defined based on their physical appearance, their genetic makeup, or their response to certain environmental conditions. Strains are often used in microbiology to study the diversity of a particular species, and to study the evolution of a particular species.

12 My Experiments

Microbe numbers shift daily in response to your environment, so a single sample won't give much more than a brief snapshot at a single point in time. Here are some of the experiments I've tried on myself, in over 600 tests since 2014. What happens in your case?

12.1 Summary of My Experiments

During the period from 2014 through early 2019, I sequenced over 600 samples of my microbiome. Inspired by the experiment in a 2014 paper by David Lawrence[1], 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. A feature that seems to be true about one person may not apply to another.

[1] David, Materna, et al. (2014)

12.1.1 Diversity

The general consensus is that diversity is good: a greater variety of microbes ensures more resilience 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 high enough that it's almost never useful to use a single result. For example, here's my diversity during a typical week: (Figure 12.1))

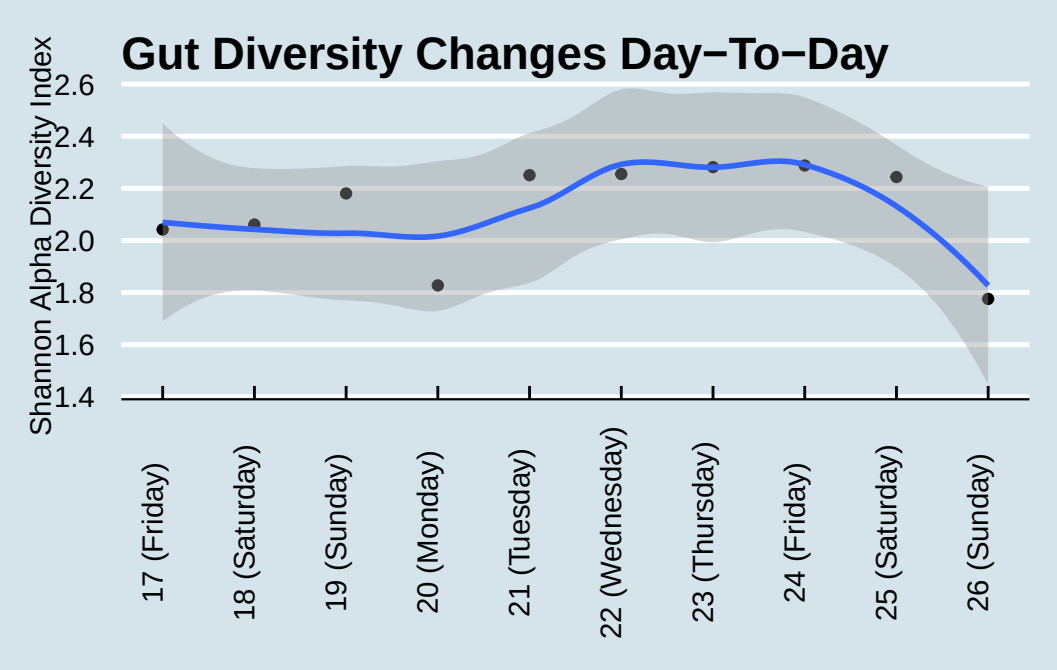


Figure 12.1: Diversity changes significantly day-to-day.

If Monday were my only test, I may have been disappointed with my 1.83 score. Wait another day or two and, with no significant changes in diet, I was up to 2.29 – before plunging to 1.78 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 (Figure 12.2))

12.2 Kefir and the Microbiome

Everyone interested in the microbiome eventually has to check out kefir. Google the phrase "one of the most potent probiotic foods available" and you'll find kefir in all the top results. A recent BBC documentary that tested people after consuming different types of "gut-friendly" foods found it had by far the biggest effect. My interest piqued when, after my disappointment with kombucha, I spoke with a man who happened to mention his good luck with kefir as a

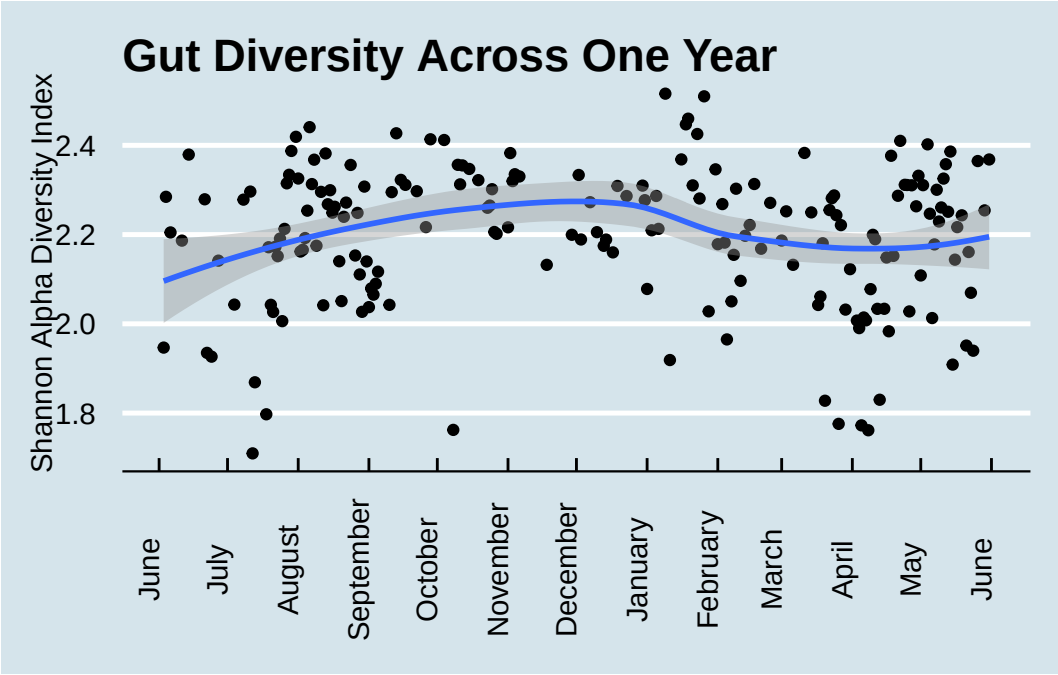


Figure 12.2: Gut diversity varies day-to-day but holds to a recognizable range within a single individual

solution to his many gut issues. On a doctor's recommendation, he tried kefir for a number of years with limited success, until — frustrated with the $3/day expense of buying it at Trader Joe's — he began making it himself at home. "What a difference!" he claimed.

Did it work for me? Yes! I found a very noticeable change in my gut microbiome — the most significant I've seen among my many experiments. Look at my daily levels of *Leuconostoc*, a prodigious synthesizer of Vitamin K known to be found in kefir. (Figure 12.3)

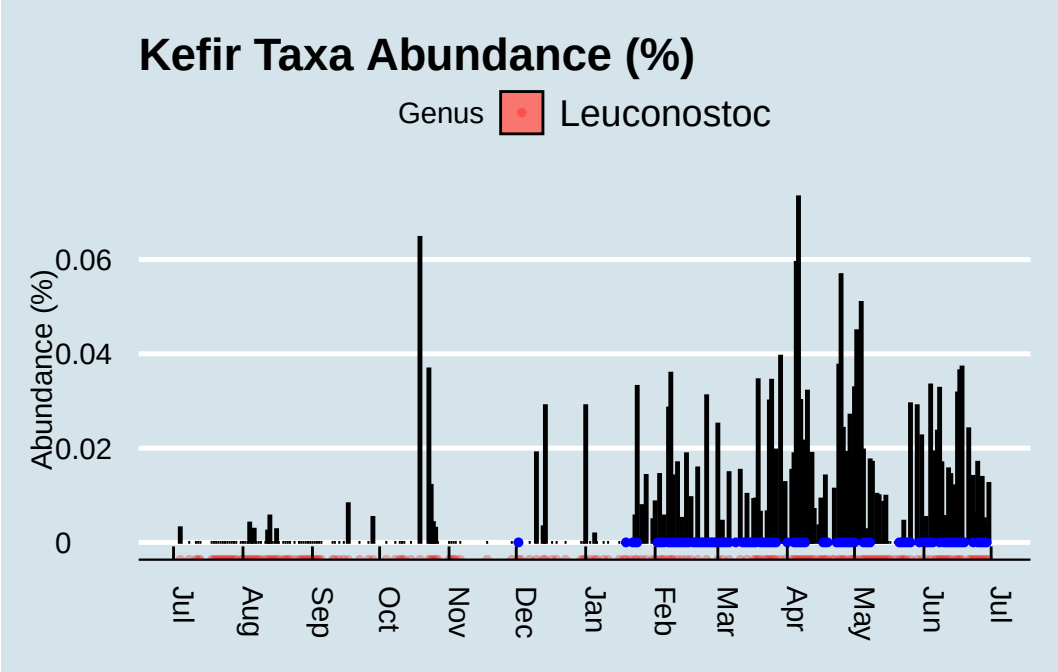


Figure 12.3: Levels of this key microbe jump suddenly when I drink kefir (blue dots)

The blue dots in the chart are days when I drank kefir. Since I sample near-daily over the entire chart, we can see that both of these taxa suddenly appeared shortly after I began to consume kefir. I had almost none beforehand. Also note that the levels seem to dip when I skip drinking for a few days, such as during my business trips out of town in mid-March and another in early-April.

So apparently it has a big effect on the microbiome. What is this stuff anyway?

The first thing to know about kefir is the pronunciation. Say "Keh-FEAR", with the accent on the second syllable, not "KEE-fur" or "kEH-fir". The Russian origin of the term is a reminder of a time in the distant past when — it's unclear exactly where or how — the first batch was prepared and then passed along, its microbial components shared from person to person until it reached today's status as a popular drink you can buy in most grocery stores.

Making it at home brings more than just financial benefits. Commercially-purchased drinks are subject to unavoidable regulatory, shelf-life, and consistency contraints that matter for

successful business, but not necessarily for nutrition. More importantly, if you believe like I do that microbes are highly-customized to our environments, making at home will ensure that the kefir is well-adapted to your own personal microbial environment. The batch that survives and thrives in your kitchen will have proven its ability to withstand whatever conditions you face there.

Making it yourself is surprisingly easy. It begins with a bundle of the component microbes, a cauliflower-shaped substance usually called the "grain" or "seed" that looks like Figure 12.4

Figure 12.4: A few of these cauliflower-shaped kefir grains will fermet a whole glass of milk

Instruction books often tell you to be careful how you handle the grains, but I find them robust enough that I pick them up with my bare fingers. I drop them into a glass of milk left I leave sitting on the counter overnight and — voila! — twenty four hours later, the liquid has turned into kefir. Pull out the kefir grains from that glass, plop it into another, and you're all set for tomorrow's batch. Unlike yogurt, which requires heating and a stable temperature, kefir doesn't appear to care how it's handled, so long as you keep it at room temperature and can wait for twenty four hours. The reaction might vary by a few hours if the room is a bit colder

or warmer, but otherwise I find it surprisingly consistent. Just set and forget.

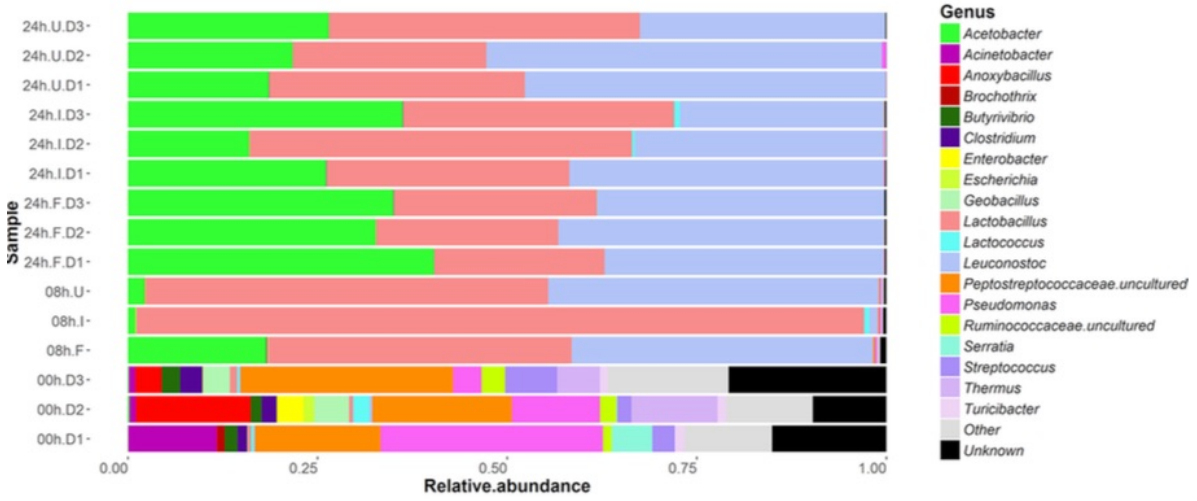
I found that the only hard part is getting started. Once you have the grains, making more kefir is easy, but where do you get the grains in the first place? It's supposedly possible to make them from scratch using a goat-hide bag filled with pasteurized milk and the intestinal flora of a sheep, but I haven't tried that myself. I'm told it works so long as you shake every hour and maintain a constant temperature.

You can order some starter grains online for under $25, but for shipping purposes the manufacturers generally give them to you in a freeze-dried form that requires a week or so of preparation before the microbes are fully alive and kicking out drinkable quantities of kefir.

I got mine by asking around until I found a neighbor who had been brewing his own. Anyone who makes homemade kefir will be happy to give you some extra grains. The fermentation process causes the grains to multiply, and you will find yourself throwing them out regularly.

The grains themselves contain a combination of lactic acid bacteria (*Lactobacillus*, *Lactococcus*, *Leuconostoc*), acetic acid bacteria (*Acetobacter*), and yeast, clumped together with casein (milk proteins) and complex sugars in a type of carbohydrate molecule called *kefiran*. The nutritional content apparently varies depending on fermentation time and other factors, but there's a lot of good stuff in there[2] (Figure 12.5).

A rigorous microbial analysis by an Irish lab [3] shows precisely which microbes are present in kefir at various stages in the fermentation process. This chart shows the composition of ordinary pasteurized milk as it changes from before adding kefir grains (time 0 at the bottom) until 24 hours have passed (top) and the milk has been transformed into just *Acetobacter*, *Lactobacillus*, and *Leuconostoc*.



[2]Otles and Otles (2003) http://files.cienciapatodos.webnode.pt/200000022-79ffe7af9e/Kefir.pdf
[3]Walsh et al. (2016). Also a 2-minute Youtube presentation

Otles and Cagindi: Kefir: A Probiotic Dairy-Composition

Table 1: The chemical composition and nutritional values of kefir (Renner and Renz-Schaven, 1986; Hallé *et al.*, 1994)

Components	100 g	Components	100 g
Energy	65 kcal	Mineral content (g)	
Fat (%)	3.5	Calcium	0.12
Protein (%)	3.3	Phosphor	0.10
Lactose (%)	4.0	Magnesium	12
Water (%)	87.5	Potassium	0.15
		Sodium	0.05
Milk acid (g)	0.8	Chloride	0.10
Ethyl alcohol (g)	0.9		
Lactic acid (g)	1	Trace elements	
Cholesterol (mg)	13	Iron (mg)	0.05
Phosphatateds (mg)	40	Copper (µg)	12
		Molybdenum (µg)	5.5
Essential amino acids (g)		Manganese (µg)	5
Tryptophan	0.05	Zinc (mg)	0.36
Phenylalanin+tyrosine	0.35		
Leucine	0.34		
Isoleucine	0.21	Aromatic compounds	
Threonine	0.17	Acetaldehyde	
Methionine+cystine	0.12	Diacetyl	
Lysine	0.27	Acetoin	
Valine	0.22		
Vitamins (mg)			
A	0.06	B_{12}	0.5
Carotene	0.02	Niacin	0.09
B_1	0.04	C	1
B_2	0.17	D	0.08
B_6	0.05	E	0.11

Figure 12.5: Nutritional content of kefir. (Source: Otles and Otles 2003)

Table 12.1: Results from sequencing two distinct types of kefir (Genus)

	Kefir1	Kefir2
Lactococcus	96.06	1.07
Leuconostoc	3.02	0.06
Lactobacillus	0.22	98.40
Faecalibacterium	0.14	0.01
Roseburia	0.06	0.00

Table 12.2: Results from sequencing two distinct types of kefir (Phylum)

	Kefir1	Kefir2
Firmicutes	99.75	99.57
Bacteroidetes	0.12	0.06
Proteobacteria	0.09	0.36
Actinobacteria	0.03	0.01
Verrucomicrobia	0.01	0.00

The uBiome test I used unfortunately can't detect yeasts, so I don't have an easy way to track the non-bacterial microbes in my kefir. But I *can* run the mixture through the same gene sequencing that I use for my other samples. I tested the kefir twice: once by simply dabbing the swab into the mixture that was waiting for me in the morning, and another swab from the same batch after removing the grain for an additional 24-hour "second ferment". This is what I found when I sequenced the kefir from two different batches: (Table 12.1)

These are the only taxa that met the 0.07% abundance criteria discussed previously. But even without that cutoff, the uBiome pipeline shows no *Acetobacter*, despite its prominence in the study shown above.

I wondered if this is simply due to the way uBiome labels the taxa that are found. Maybe the label *Acetobacter* just isn't often assigned to uBiome samples. When I checked, I could find none in any of my own samples or of the hundreds of others that people have sent me. What's more, none was reported in a large population study[4] either. So apparently it just doesn't show up often in humans, though I wonder why it wouldn't show up in the 16S sequencing of my kefir sample.

The answer, according to the uBiome scientist I talked to, is that *Acetobacter* is too similar to other genera for it to be accurately distinguished with a 16S test. So if we can't see at the genus level, let's look at a higher level, such as phylum. Table 12.2

Because *Acetobacter* is within Phylum *Proteobacteria* and Order *Rhodospirillales*, we would expect to see some of those microbes if any of it were present. Looks like my kefir doesn't include anything remotely resembling *Acetobacter*.

[4]See Zhernakova et al. (2016)

That's what's in the kefir grain itself. How does regular drinking affect my gut microbiome?

To find any taxa that may have suddenly changed as a result of kefir-drinking, let's look at a heatplot that shows the relative abundances of all my top microbes over time. Darker spots are days when I have *less* of a particular bacterium, lighter spots are days when I have more.

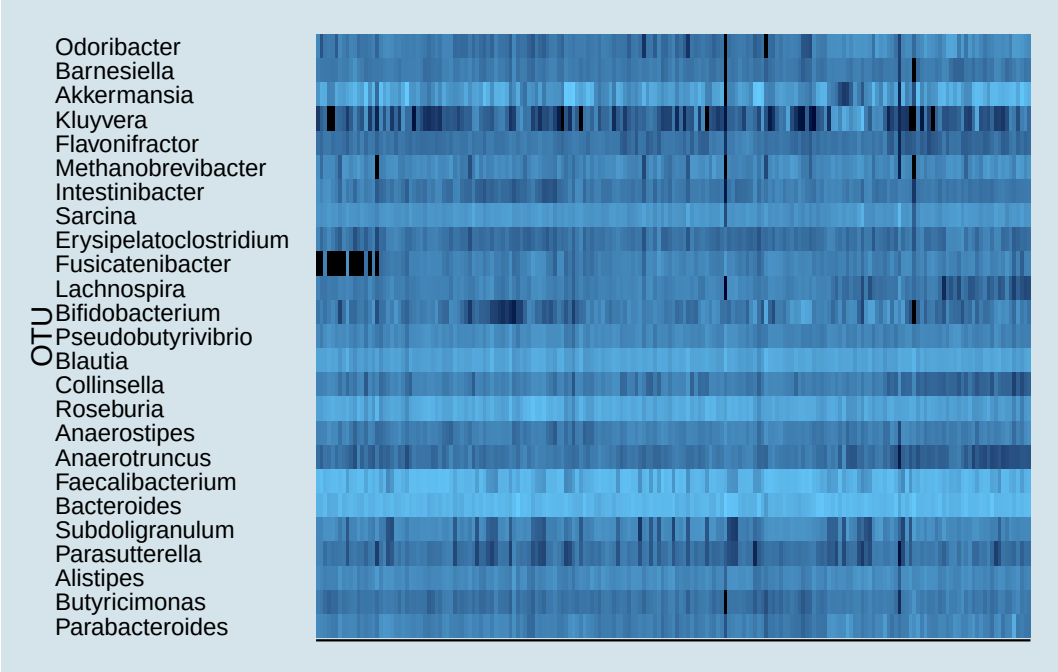


Figure 12.6: Daily abundances of each microbe over time.

Note the sudden appearance of the genus *Fusicatenibacter*. You rarely see such a dramatic and consistent change as a result of an experiment, but unfortunately, little is known about this genus. A member of the Clostria class of phylum Firmicutes, an internet search reveals little of interest. But it definitely appears in my samples after drinking kefir.

In fact, look how the levels appear to coincide precisely with the periods when I drink kefir:

This is especially interesting because the only previous date when my gut saw any of this taxa was in December – on another occasion when I drank some kefir. In fact, *Fusicatenibacter* is such a strong predictor of kefir drinking that I can use it as a way to look back in time to see the samples when I drank some.

How common is *Fusicatenibacter* in gut microbiomes? Here's a density plot look at a few hundred samples collected from other people.

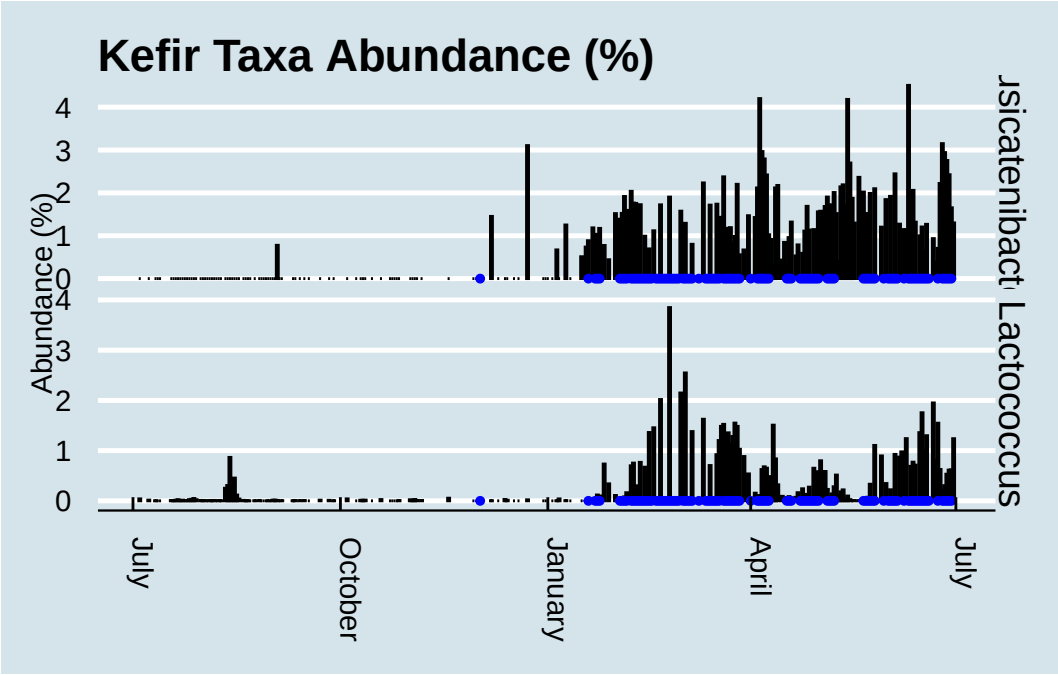
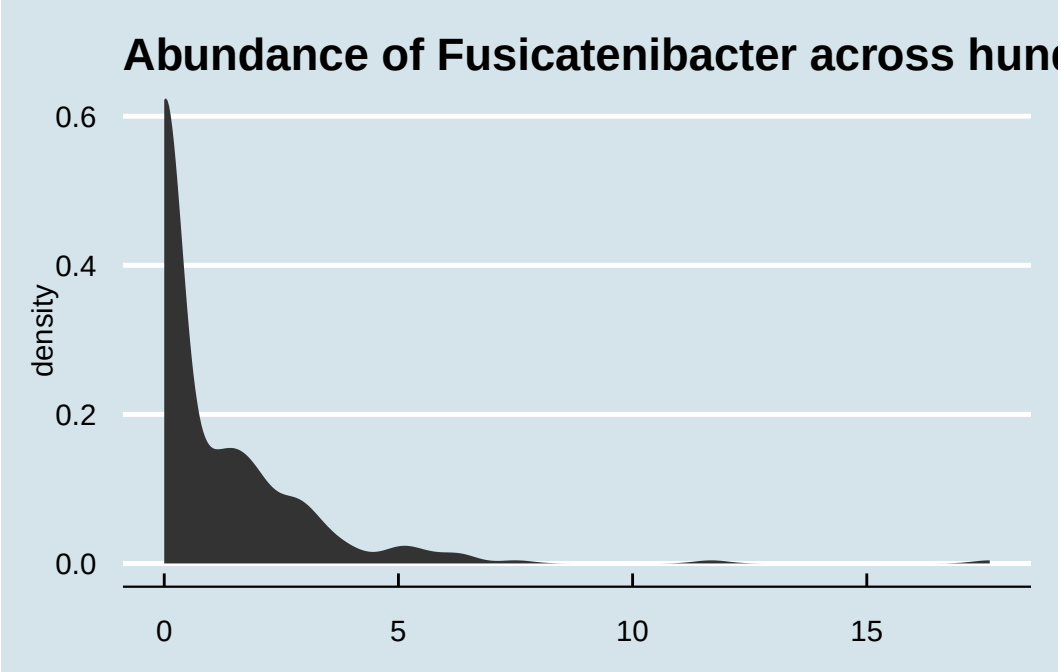


Figure 12.7: Abundance of two important genera over time. Blue dots are days when I drank kefir



Although most people have none, it's not unusual for people to have a few percentage points of *Fusicatenibacter* regardless of whether they regularly drink kefir.

But other than this clear change in my gut microbiome, did I notice any differences in health?

Here the answer is more ambiguous. As a healthy adult, I don't have any particular "problems" I'm trying to solve. I remained healthy during the period of the experiment, so the kefir certainly doesn't appear to have made anything worse. My sleep hasn't substantially changed either, and although I'm generally pretty even-tempered, I didn't notice any particular changes positive or negative in my mood either.

The one area where, subjectively, where I feel different is in my overall sense of energy. Although I can't put my finger on anything quantitative, I *do* notice that I seem to be a little more energetic on days when I drink kefir. Measuring that more precisely may be a good followup test.

12.3 Kombucha

For healthy bacteria-rich drinks that affect the microbiome, many people immediately think of kombucha. Served chilled during the summer, it has a well-deserved reputation as a natural refreshing alternative to soft drinks. Despite its tangy, mildly sweet taste, it has a surprisingly

low amount of sugar: only six grams in a serving[5], compared to more than 20 grams in the same amount of orange juice or 39 grams in a can of Coke.

The sugar is missing because it's been eaten by microbes, a complex blend of bacteria and yeast that convert regular tea (usually black, but also oolong or green tea) into a complex, flavorful beverage. The fermentation process is ideal for adding other ingredients for taste, so there is no end to the interesting flavors possible, giving rise to a highly competitive commercial market: U.S. supermarkets sold $180 Million of the drinks in 2015.

Kombucha fermentation begins with a SCOBY, a "Symbiotic Colony of Bacteria and Yeast", a pancake-sized disk-shaped gelitintous object also known as a "mother" or "mushroom", which it sort of resembles. Despite the nickname, the only funji in the SCOBY are yeasts, combined with a complex blend of bacteria and other single-celled microbes from many parts of the tree of life. The different microbes need one another to produce the distinctive sweet and fizzy taste. Yeast cells convert sucrose into fructose and glucose and produce ethanol; the bacteria convert glucose into gluconic acid and fructose into acetic acid; caffeine from the tea stimulates the entire reaction, especially the production of cellulose by special strains of bacteria.[6]

There have been many anecdotal claims of the effect of kombucha on health, purporting benefits ranging from better eyesight and thicker hair to cures for various diseases, though not everyone thinks it's healthy. Even some alternative health experts, like Dr. Andrew Weil, recommend against it. Many of the claims for and against kombucha have been studied experimentally, in mice as well as humans, often with compelling results, but I'm unable to find any good data showing how it affects the microbiome.

So I tested it myself.

For seven days, from July 27 to August 2, I drank 48 ounces per day of commercially-purchased GT's Gingerade Kombucha. That's three full bottles, or six servings a day for a week.

The key bacteria in the SCOBY are from phylum *Proteobacteria* and order *Rhodospirillales* of acetic- and gluconic-acid producting microbes that include genus *Gluconacetobacter*, closely related to *Acetobacter*, the key to the fermentation of vinegar. Thanks to the action of these microbes, kombucha is quite acidic, between 2.5 and 3.5 pH, almost as acidic as the 1.5 or 2.5 of a healthy stomach. These bacteria apparently don't survive ingestion. They are rarely, if ever, found in human guts[7], so whatever effect, if any, they have on the microbiome is indirect.

The label claims each bottle contains one billion organisms of two microbial species. The first, *Saccharomyces boulardii*, is a popular "healthy" microbe, well-studied and proven as a safe digestion aid. A close cousin of brewer's yeast, its cell wall tends to stick to pathogens, which may account for its proven ability to prevent and fight diarrhea.[8]. Unfortunately, it is not a

[5]Though one large test by the BevNet industry trade site says the labels may under-report the real amount,and in 2017 a judge approved a settlement to a class action claiming misleading sugar content by one manufacturer

[6]The best scientific review I know is in Dufresne and Farnworth (2000)

[7]For a list of microbes that *are* found in the gut, see: http://www.raeslab.org/companion/vlaams-darmflora-project.html

[8]https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4542552/

bacterium, and so won't be detectable in my 16S-based microbiome tests.

The other added species *Bacillus coagulans* is often found in human guts, and should be easy to find. The specific one used in GT's drinks is the patented Bacillus coagulans GBI-30, 6086, a particularly hardy spore-forming microbe that can survive boiling and baking. Because it's well-studied and safe, it's a popular "probiotic" food additive and appears to have some beneficial effects on digestion.[9]

I tested my gut microbiome each day, as well as my mouth and skin microbiome at intervals during the experiment and sure enough, the *Bacillus* shows up loud and clear. (Figure 12.8)

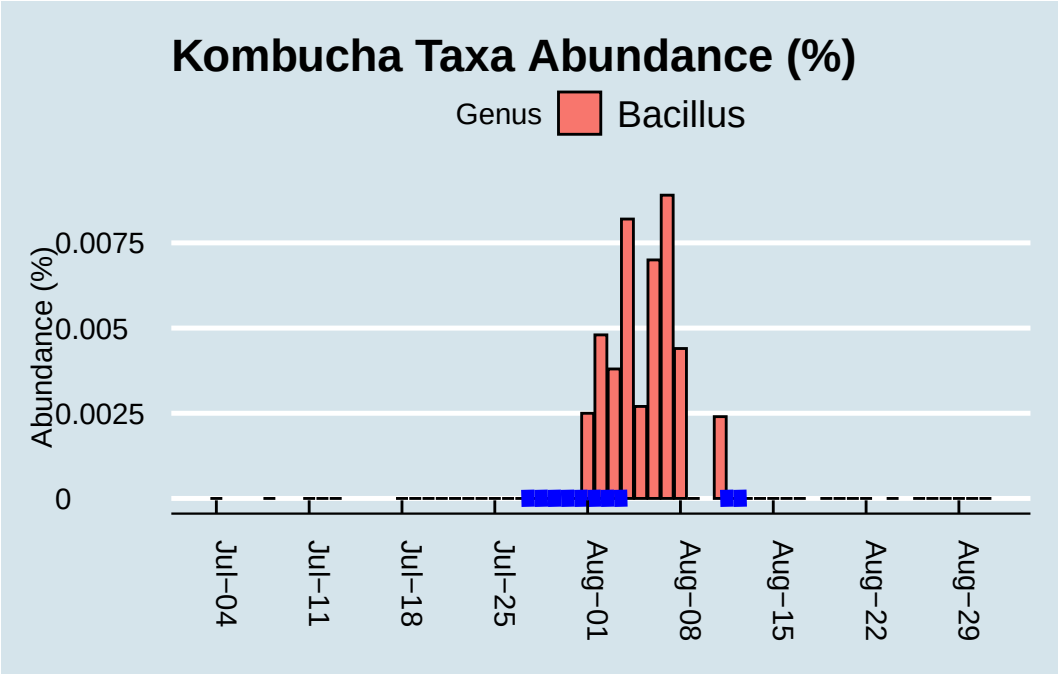


Figure 12.8: The blue line represents days I drank 6 servings of kombucha.

It took a few days of heavy kombucha drinking, but eventually those microbes became detectable. Given the known hardiness of *Bacillus*, this isn't necessarily all that surprising. Still, it's a nice confirmation that the test works; after all, in my hundreds of daily tests, I see this microbe only in the few days after drinking this brand of kombucha. But maybe the *Bacillus* just comes in and out, safely protected as a spore, without really influencing my microbiome. Can we see evidence the kombucha affected something else about my microbiome?

Diversity doesn't seem to change (Figure 12.9). I looked at the overall mixture of microbes and abundances using the Shannon diversity metric, commonly used by ecologists to tell measure the richness and variety in an environment. Don't let the scale of this graph fool you:

[9] https://www.asm.org/index.php/general-science-blog/item/6761-bottoms-up-discover-the-microbes-in-probiotic-drinks

I set it narrowly to see precisely how diversity changes each day. A Shannon diversity change of a tenth of a point or so, as in this graph, is pretty trivial.

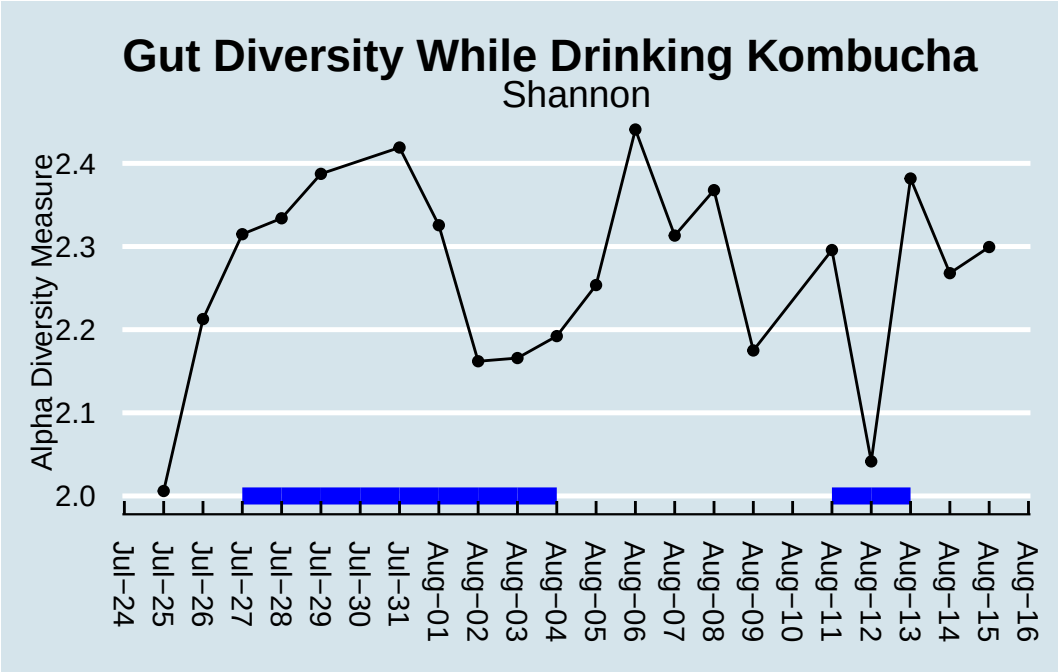


Figure 12.9: How my overall family-level diversity changes while drinking kombucha. I drank 6 full servings on each of the days marked with the blue line.

Diversity had been climbing before the experiment began, so I don't think we can lay that initial increase on kombucha. Incidentally, had I not been testing daily, I might be tempted to say diversity *decreased*. This is something that makes me skeptical of the results of many scientific studies: the microbiome flucutates so much day-to-day that what you see is very dependent on *when* you test. (By the way, note that the July 30 sample is missing, due to a failure in the lab processing.)

Let's look at that order *Rhodospirillales* that contains the genus *Acetobacter* found in the SCOBY. (Figure 12.10)

If we squint enough, we might credit that large spike with kombucha drinking. It's possible, but then how would you explain the crash the following day, or the other apparent spikes in other parts of the chart? I conclude it's probably a coincidence. More than likely, microbes like this from the SCOBY itself are not in the beverage anyway.

What about other microbes? Here is a heatmap showing the changes of the top 20 genus during my experiment (Figure 12.11)

I don't see any patterns. Usually, if the experiment causes a change, I'll see an obvious streak from left to right somewhere in the heatmap, but I don't see that.

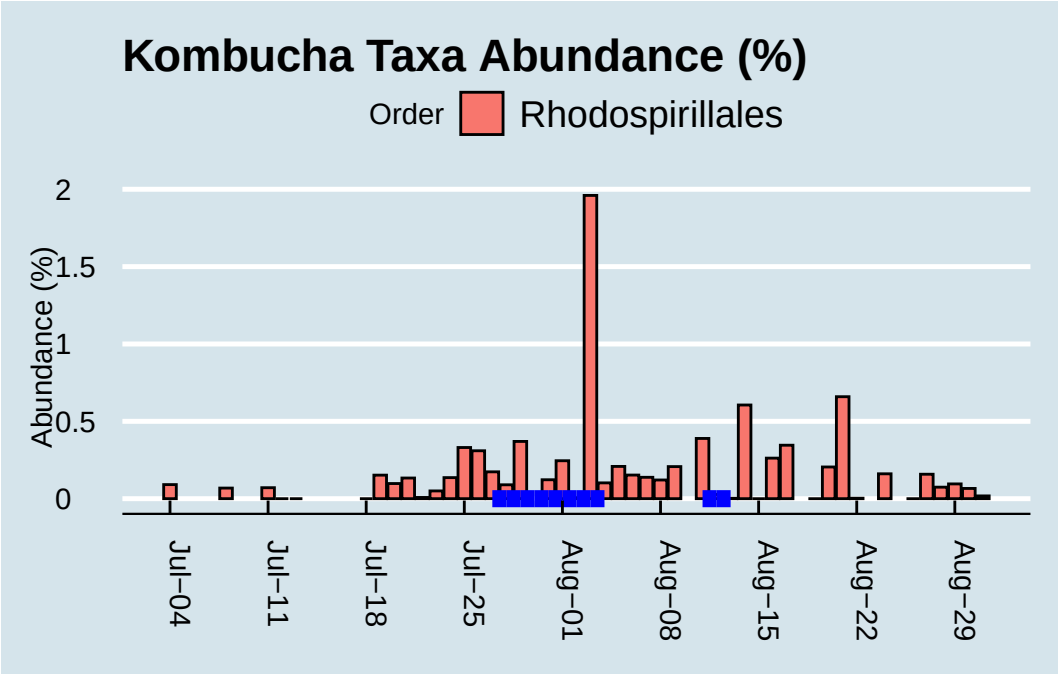


Figure 12.10: Abundance of microbes that include the genus Acetobacter found in the SCOBY

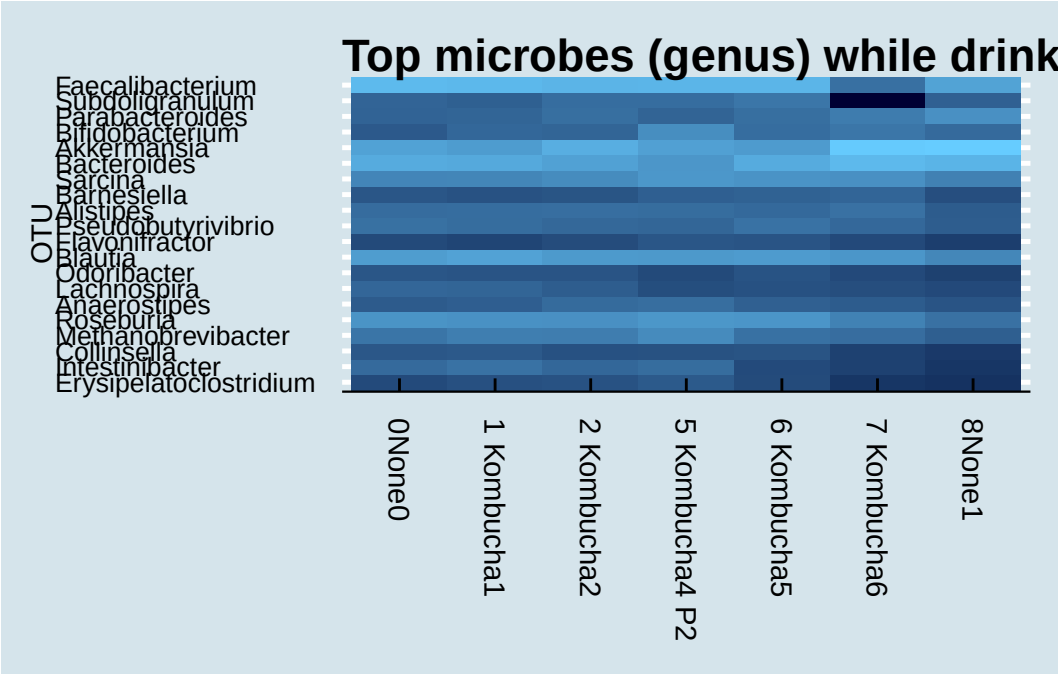


Figure 12.11: Top 20 changes during my experiment

Finally, let's look at the levels of a few "probiotic" microbes, including the one listed on the label (Figure 12.12)

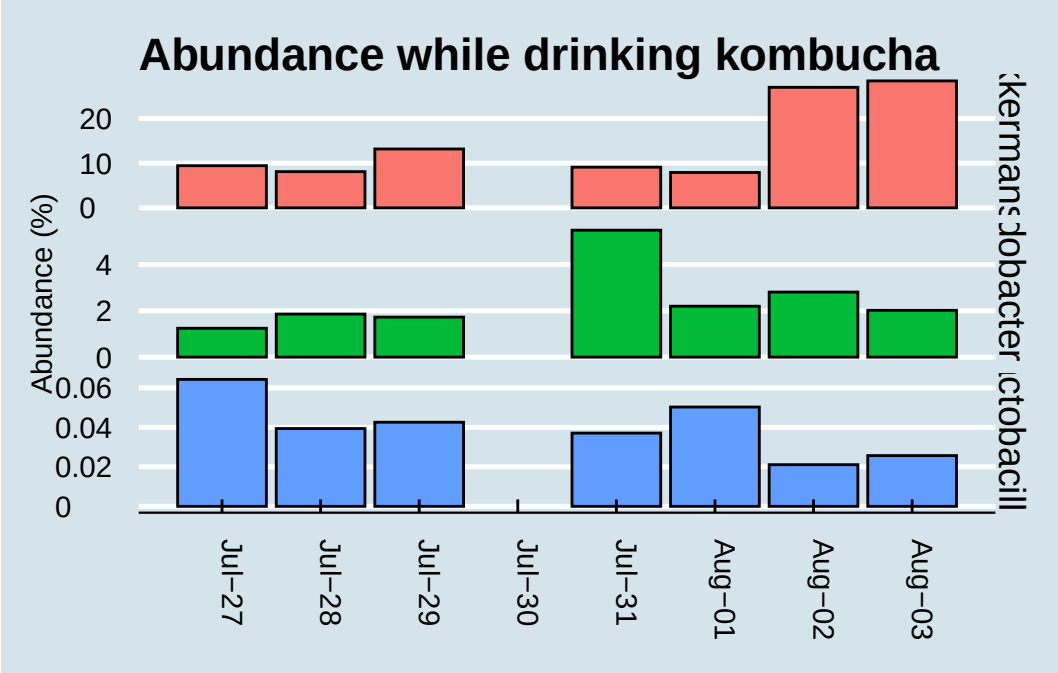


Figure 12.12: Abundance of key 'probiotic' microbes while consuming kombucha.

While *Akkermansia* seems to rise near the end of the sequence, it's hard to see any real patterns here.

For comparison, let's look at a longer time horizon (Figure 12.13)

Although we can't positively credit kombucha for that spike in *Bacillus* during my experiment, it's interesting that I had none of it in the weeks beforehand, and that it disappeared again in the weeks afterwards. I drink this brand of kombucha occasionally, and yes the same microbe shows up occasionally too, sometimes a few days afterwards.

In my years of testing, I rarely see *Bacillus* in my gut microbiome, but the few times when it *does* appear, there seems to be a relationship to drinking the same brand of kombucha a few days beforehand. There are also times when I drink kombucha and *don't* detect this microbe, so the association isn't perfect, but then again this was the only time I had so much of this brand all at once.

My conclusion: when consumed in large amounts, GT's Gingerade Kombucha leaves new *Bacillus* microbes in my gut. Although they don't appear to stick around permanently, the association is strong enough that I bet it works in you too. Other microbes, including so-called "probiotic" ones, don't change much at all.

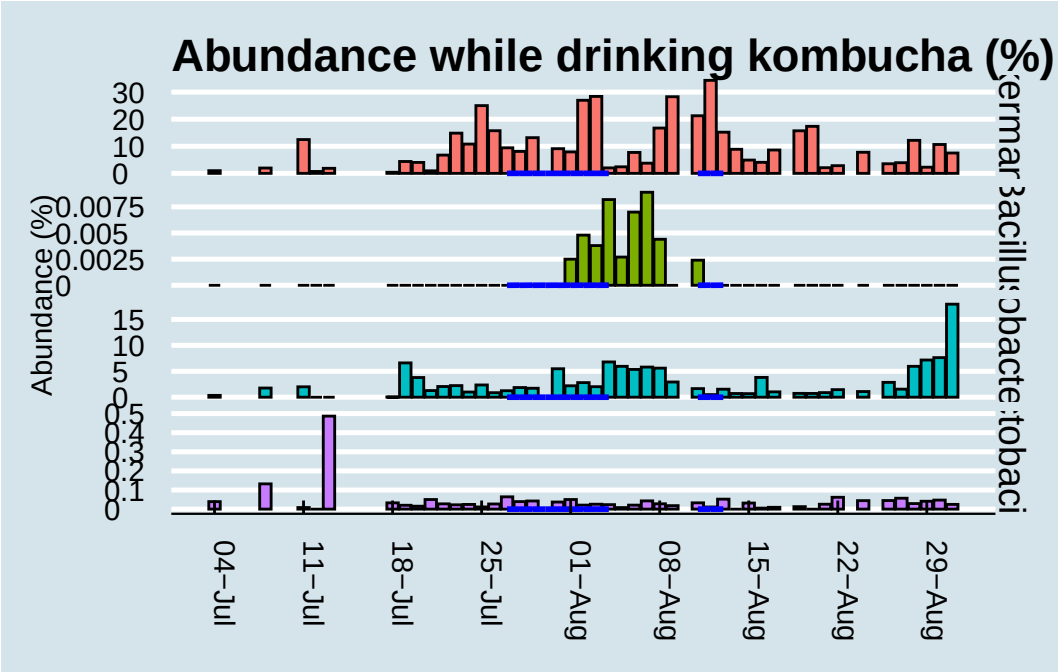


Figure 12.13: Daily abundance of key microbes while drinking kombucha (blue lines). Blank regions are days when I have no data.

But don't take my word for it. The full dataset and analysis tools are on Github: https://github.com/richardsprague/kombucha

There is much more analysis that can be done with this data. Some of the ideas you might try are:

- Study correlations among the taxa. Which ones are correlated, and which are not?
- Which taxa appeared and/or disappeared during the experiment?
- Is there a relationship between the microbes known to be present in kombucha and those in any of the gut results?
- How do these results compare to *you* when you drink kombucha?

Please study as much as you like, and let me know what you find!

P.S. The term "kombucha" is an unfortunate mistranslation of a Japanese word that means "seaweed tea". A fermented version of seaweed tea exists, but it has nothing to do with the drink described here.

12.4 Traveling to China

During my other international travel experiments, I tested only twice: before and after. Did I miss anything by not testing daily?

On a recent trip to Beijing, China, I took enough kits to test myself every day: gut, skin, nose, and mouth.

Any travel presents major challenges to the microbiome. Besides the significant differences in food, you are surrounded by different people (and germs) and weather. A trip to China involves a 12 hour plane flight too, exposing the body to a long period of lowered air pressure, tight quarters with people and recycled air, and of course the jet lag that accompanies a fifteen hour time shift. With all of that, it would not be surprising to see a significant shift in my microbiome.

Here's an overall heat plot of my gut, day-to-day before and after the trip. Figure 12.14

I wasn't surprised to see the rise in *Kluyvera*, a genus that on the 16S test can include sometimes-pathogenic species like *E. coli* or *Shigella*. These microbes can go up and down regularly, sometimes for no apparent reason at all, but often due to a significant change in environment, like on a trip where you're exposed to many new microbes.

But the obvious standout is the genus *Coprobacter*, which soared beginning a few days after arrival and settled back after my return. I looked in my other samples over the long term and find that it is strongly associated with my China trip. (Figure 12.15)

Among my years of daily sampling it appears to have bloomed only once – this trip – after which it settled back to its quiet little self. The very first time I noticed any at all was early

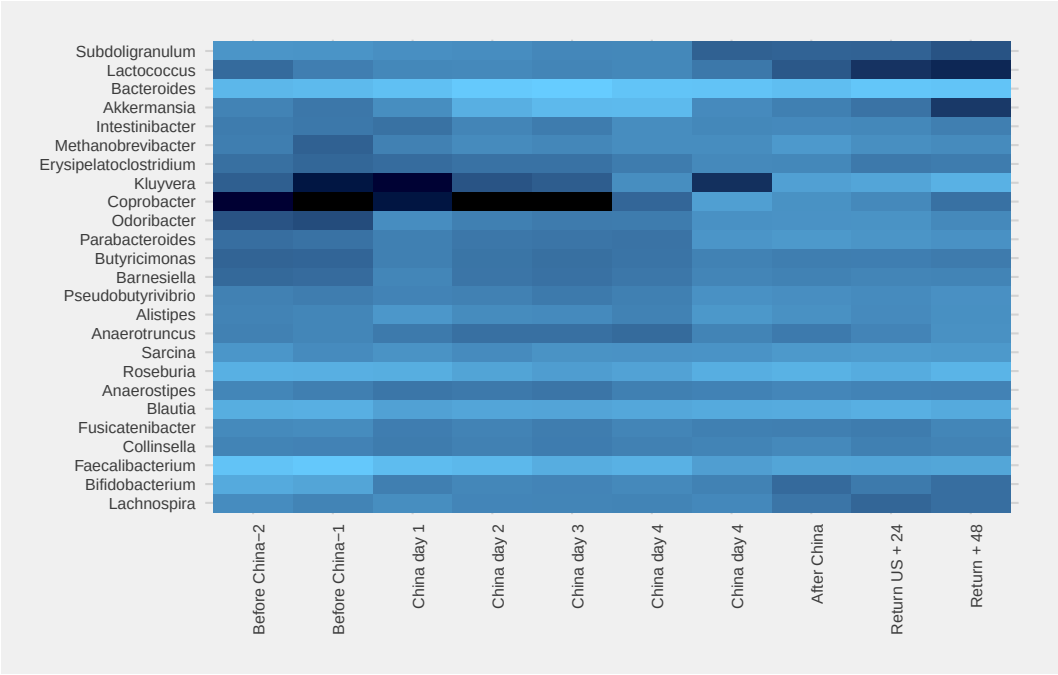


Figure 12.14: Gut samples before/during/after a trip to Beijing from the US. Dark is 0, lighter colors are higher abundance

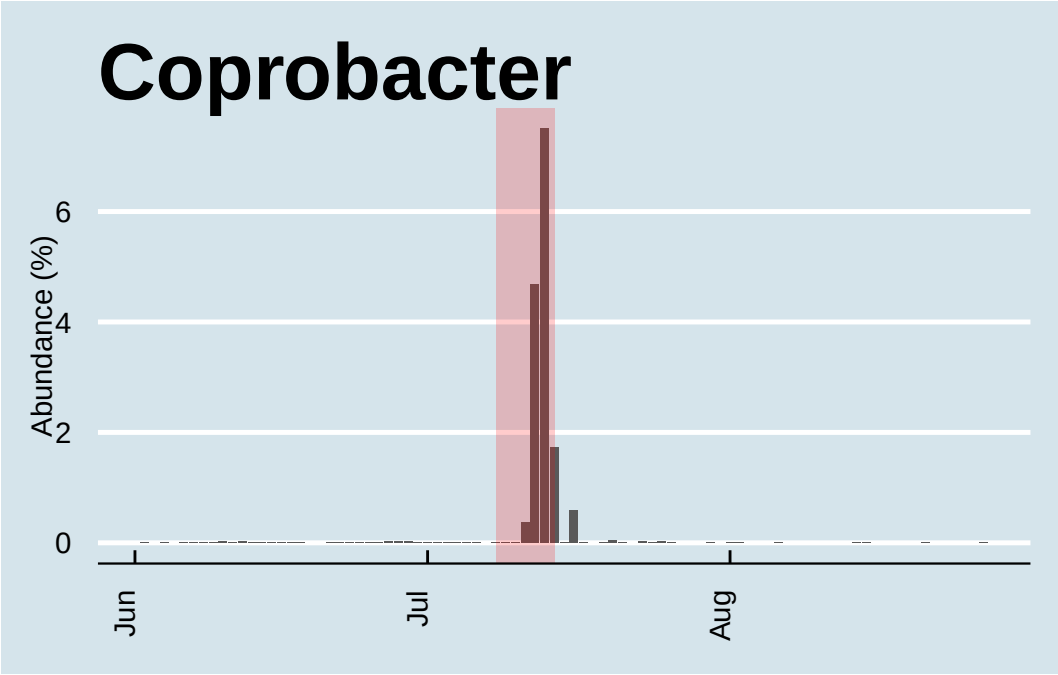


Figure 12.15: Changes in gut microbiome abundance of *Coprobacter* over time. Area shaded in red is the period while traveling from the U.S. to China.

in the year, coincidentally (?) after I began drinking kefir. But even then, the amounts were tiny (under 0.01%) and often zero – until this trip.

When I looked at the hundreds of other samples people have sent me, I could find *Coprobacter* in just a few, and then only at relatively small levels (under 0.4%), less than a tenth of what I found on my biggest day (4.9%). The big 4000+ person Zhernakova study[10] found it in small amounts in many people, but again, not very much. I couldn't see any obvious patterns in any of the samples: some were from heavy travelers including some who had been to China, some not; some were from healthy people, some not. I found small amounts in a few skin samples (including my son, in a sample taken shortly after my return), but always in small amounts and with no clear patterns.

The natural question to ask about this microbe is *what does it do?* Unfortunately, as in so many of these cases, even Dr. Google can't tell me much besides a few passing references in hundreds of top academic papers. It doesn't seem to be a well-studied microbe. I know that it's a member of the *Bacteroidetes* phylum, a "rod-shaped, gram-positive, obligate anaerobe", and my particular species appears to be *Coprobacter fastidiosus*.

The Russian scientists who first isolated it (in 2013[11]) found that, when cultured on glucose, it generates propionic, acetic, and succinic acids. If you look up what those acids do, you can invent lots of stories that might explain why it might appear on a trip to China (smelly? maybe the food! retinal modulation? maybe from the smog!). But I've been around the microbiome block enough times to know that you can explain just about anything if you try hard enough and you don't care about proving it scientifically.

Travel is often good for *Proteobacteria*, another large family of microbes that changed on this trip. Whenever I see high levels, in myself or others, I usually find that the body is undergoing some kind of challenge – often as a result of exposure to something unhealthy, like a sick person or bad food, and sometimes accompanied by symptoms like an upset stomach or fever. Are the symptoms a *result* of the higher levels of *Proteobacteria* or a *cause*? Maybe this phylum contains plain old pathogens, which would explain the rise in abundance, or maybe – and I'm speculating – it rises as a natural defense to *protect* us against?

Look how my levels rose (Figure 12.16). Shortly after returning home, I was on another plane, for a week in the Midwest. All that travel appears to have kept my *Proteobacteria* levels high. (Unfortunately I'm missing a few samples during that period, but I think you can see the trends). I was never ill during my trip – at least not with symptoms I could feel – but my previous bouts of illness almost always coincide with a bump in *Proteobacteria*, so who knows.

How about diversity? Did that change? (Figure 12.17)

You can see that some sites may have changed more than others. (Figure 12.18)

[10] Zhernakova et al. (2016)
[11] Shkoporov et al. (2013)

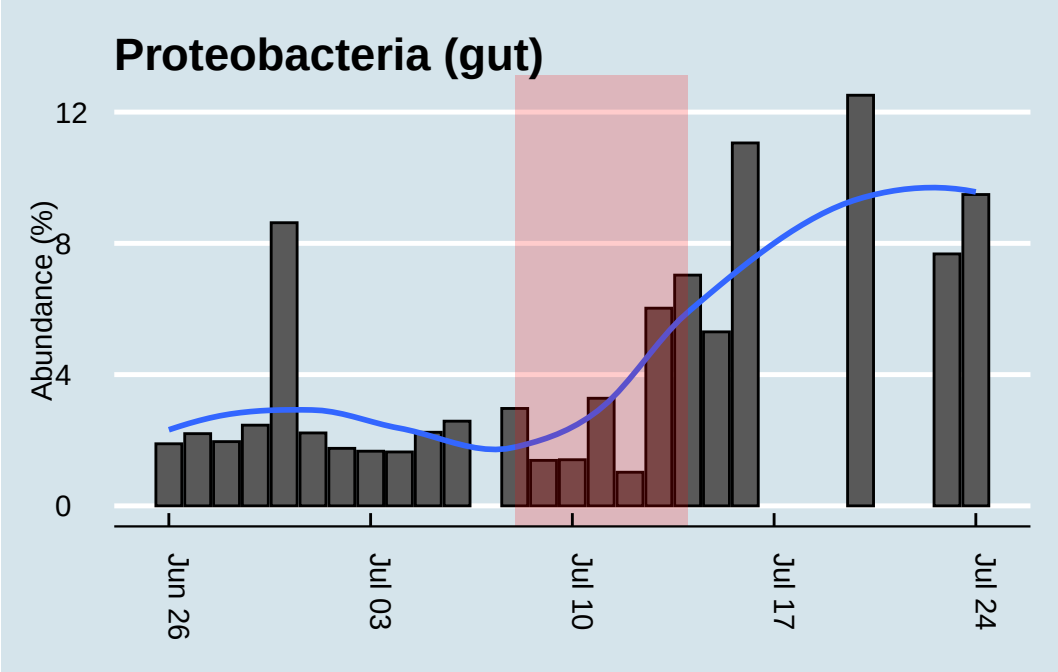


Figure 12.16: Gut Proteobacteria abundances rose during a period of heavy travel. Note: zero abundances are days with no samples available.

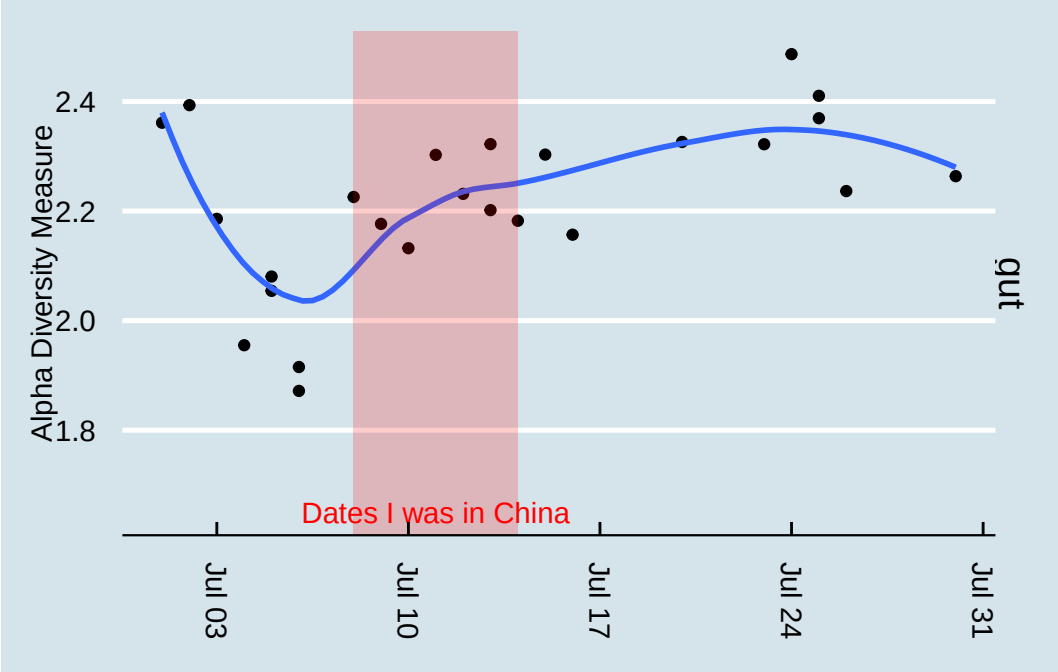


Figure 12.17: Shannon diversity of gut samples during a week-long trip to Beijing.

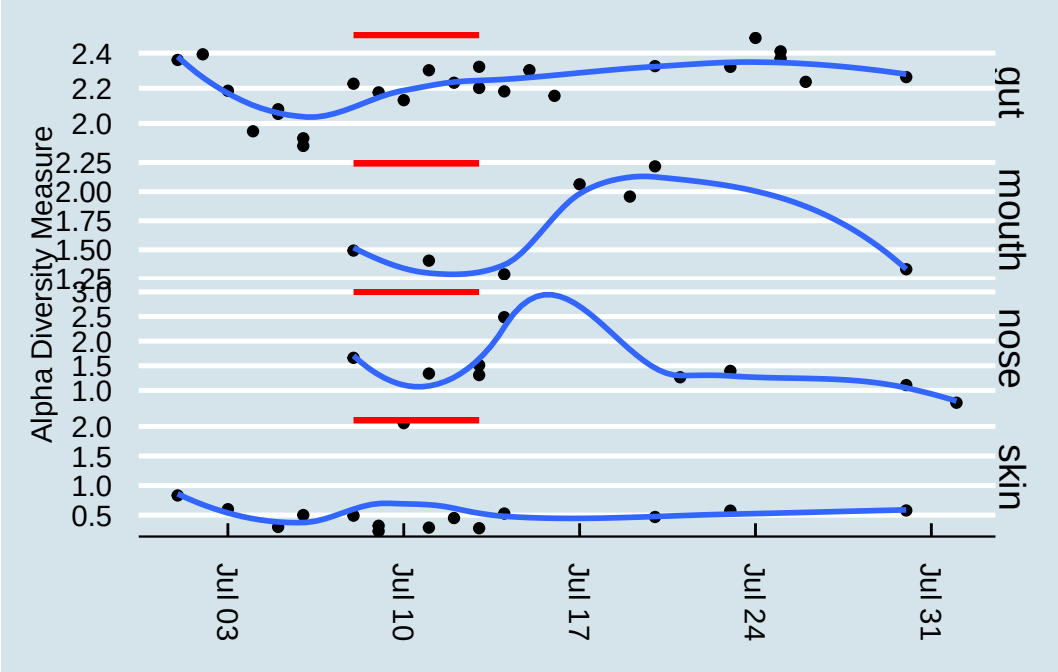


Figure 12.18: Shannon diversity of all samples during a week-long trip to Beijing.

Answer: maybe. As always, my diversity seems to bounce up and down for no apparent reason. It's not surprising that exposure to an all-new environment might tend to bring out new microbes too, as in the case of *Coprobacter*. In this case, at least as measured at the family taxonomic rank, there *was* a slight shift upward in diversity. Incidentally, on the dates after the red line, I stopped at home for a day or two and then continued on to another part of the United States. That's a lot of travel, so it wouldn't be surprising for it to have an effect on my microbial diversity.

It was a similar story with the diversity of my mouth, nose, and skin: if I *really* wanted to imagine that a visit to China caused a change in diversity, I could point to a few samples that seem to make the case, but inevitably diversity shifted again soon afterwards with no apparent cause.

I could find no other clear pattern of change in any of the other sites. When I looked through each of the individual taxa, none of them

Let's look at the other sites I tested. Did anything unusual happen in my skin microbiome? As usual, I look first at the overall heatmap to spot any obvious changes. (Figure 12.19)

One of these, *Rhizobium* is fairly abundant during the week or two before my trip, but then seems to disappear a few days after my arrival, only to bloom again right afterwards. (Figure 12.20) Interestingly, *Rhizobium* is a important nitrogen-fixing microbe found in soil, always near a plant host. What might that have to do with China, or with international plane travel?

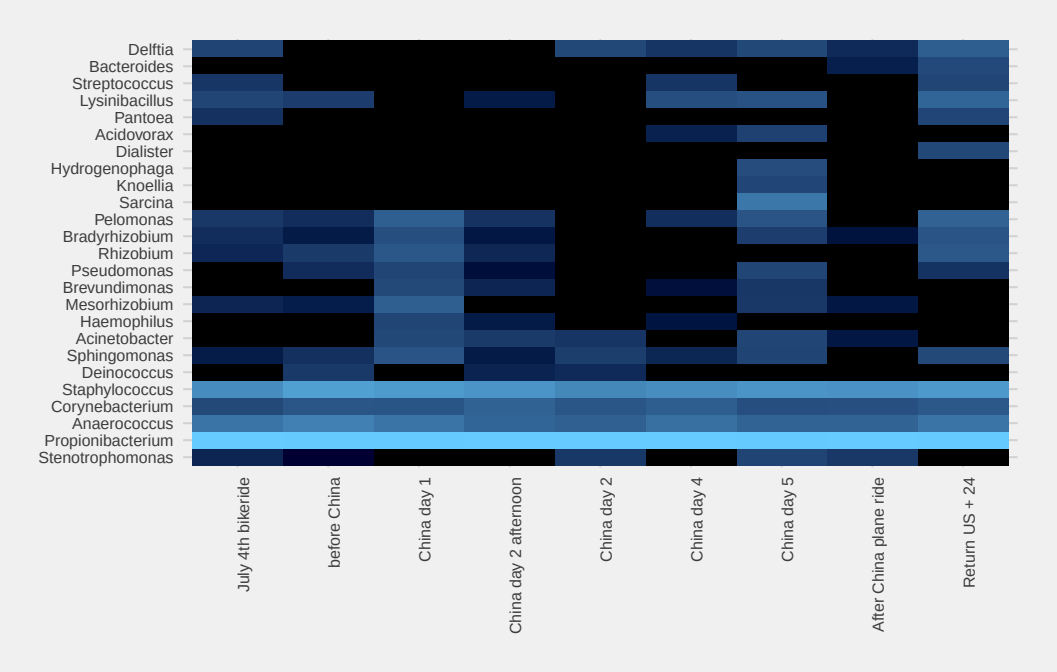


Figure 12.19: Heat map of my skin microbiome before and after a trip to China.

I looked more closely at the abundance of this microbe over time and found that it appeared in my skin only once before, during an extended visit to the northeastern U.S. Like the China trip, it happened over the summer when I typically spend more time outdoors and have greater contact with the soil. Another spike happened right after a camping trip, which makes sense.

The change in *Rhizobium* abundance this time was indeed unusual, because it seems to not have been related to anything unusual on my part. It was nice weather and I went hiking a few times during that period, but there were many other times I went hiking that didn't see a bloom in this microbe.

My conclusion: international travel to a very different place, like China, causes *some* changes to the microbiome, as you would expect. There is at least one gut microbe, *Coprobacter*, whose bloom seems highly correlated to this particular trip. I could find no other major changes that could be pin-pointed to travel this way, although the extensive testing I did for this experiment let me notice another microbe, *Rhizobium* whose unexpected rise seems to have occured just before the trip and continued to show up now and then afterwards.

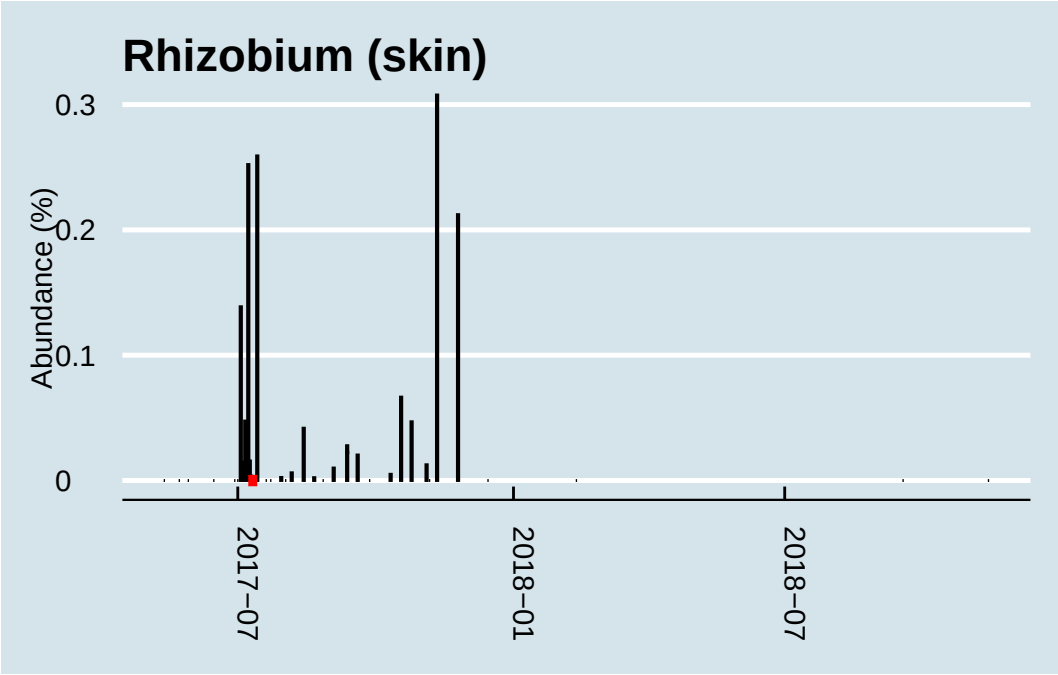


Figure 12.20: Long-term abundance of Rhizobium in my skin microbiome. Red line indicates the period of traveling to China.

12.5 Do Probiotics Work?

Probiotic supplements are a $55 billion business, with food and beverages accounting for almost 80% of that, according to an August 2021 report by Grandview Research. With unregulated health claims that range from the benign ("helps digestion") to the fantastic ("A miracle cure!"), do they make a significant difference in my own gut microbiome? I tested myself to find out.

Among unhealthy people, there is evidence that, under a doctor's care, probiotics can help with antibiotic-associated diarrhea and similar conditions in children or among people recovering from *C.* difficile infections.

On the other hand, a recent scientific review of all well-done studies of probiotics among healthy people couldn't find evidence that probiotics made much difference compared to a placebo in randomized controlled trials. When the data-heavy web site FiveThirtyEight did a week-long series on Gut Science, including a detailed survey of what's known about probiotics, they concluded: "There's no evidence in humans, however, to support taking probiotics just to generally improve your gut health."

A literature review by the US Agency for Healthcare Research and Quality found no safety issues in healthy adults, but there is surprisingly little research to show that the pills actually do anything. The independent lab Labdoor tests most common brands to see which actually contain the organisms claimed on the label, but I couldn't find anyone who tests whether the body can absorb them or not. There have been a few peer-reviewed studies showing that *some* microbes in supplements can make it to the gut[12], but these studies almost feel like special cases, where they try lots of microbes and only a few make it. It's not clear that organisms in a typical off-the-shelf bottle of probiotics have ever been tested that way.

12.5.1 Dangers

Like anything you put into your body, you can't just assume it's all upside.

Presumably you're reading this because you are convinced that microbes have a powerful affect on the body, perhaps as powerful as prescription drugs, yet you wouldn't consider taking random prescription drugs just to see what happens. The billions of microbes you send into your gut is in a concentration and quantity far greater than anything you'd get from nature. Please remember that.

Here's an analogy: let's say scientists discover a breed of parrot that is found in abundance in healthy ecosystems in Costa Rica, so they decide to introduce it to Yellowstone Park. They dump thousands of live parrots all over the park and when they count the overall species diversity the following day, they note with pride that the experiment worked: Yellowstone is now home to a new species, one that is associated with healthy ecosystems! Unfortunately,

[12] *L. reuteri DSM 17938* and *L. rhamnosus GG* in Dommels et al. (2009)

upon testing again a week later, they learn that the parrots are gone. What happened? You and I can laugh at the idiots who thought they could transplant a tropical species into Wyoming, but maybe that's exactly what you're doing if you try to introduce a new species that is not adapted to your microbiome. It may show up in a couple of early gut tests, but if it disappears soon thereafter, was it helpful at all? In the parrot example, it may actually be harmful if it served as food to dangerous predators.

Fortunately, the body is pretty robust and it's harder to deliberately change the microbiome

12.5.2 My Tests

I'm especially interested in learning whether the probiotics in the supplements actually "stick" in my gut. Taking so many billion organisms in pill form all at once may very well show up in a single gut test result, but how do I know they're not simply being flushed out of my system? Or worse, how do I know I'm not just crowding out something more important?

To find out, I tracked my microbiome daily while taking a high quality probiotic supplement, one that I received directly from the manufacturer. To be a fair test, one worth publicizing the brand name for better or worse, I'd want to try it out on multiple people, at multiple times. Because I didn't do that this time, I won't name the product other than to say that it's from a "good" brand and well-recommended.

I took the supplement once per day for nine days. I would have continued for an even ten, but I was starting to feel uncomfortably bloated those last few days. While that's an encouraging sign that the pill is working, I didn't want to do anything to seriously mess up my gut. I'm doing this experiment for fun, and it won't be fun if I get sick as a result.

Let's look for at the overall abundances for the two genera that were in the supplements: *Bifidobacterium* and *Lactobacillus*. (Figure 12.21).

The red dots represents days when I took a gut sample after consuming the probiotic. Unfortunately, despite taking and submitting samples daily, several of my results just didn't have enough reads to be useful. This chart shows only the days when I have a sample of at least 10,000 reads.

Even with that caveat, it's hard to see clear-cut evidence that the pill had a significant effect. Yes, I have slightly more of those two taxa by the end of the experiment, but seriously, not *that* much more.

Let's look at a longer time horizon (Figure 12.22).

Hmmmm, it seems the levels of those particular genera *did* increase a tiny bit at the end of the experiment, but there are plenty of other times on the chart where I see spikes too. In fact, the biggest increase happened in September when I was living it up in New Orleans, eating red beans and rice – and no probiotic pills.

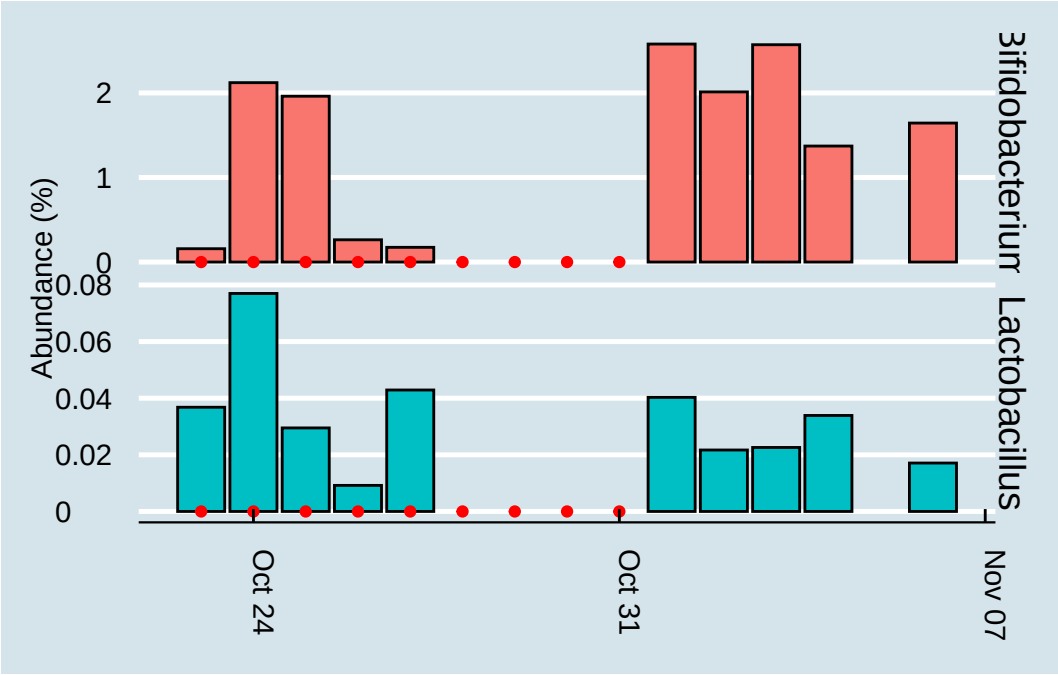


Figure 12.21: Percent abundance of key microbes (genus-level) found in the gut while taking a probiotic supplement.

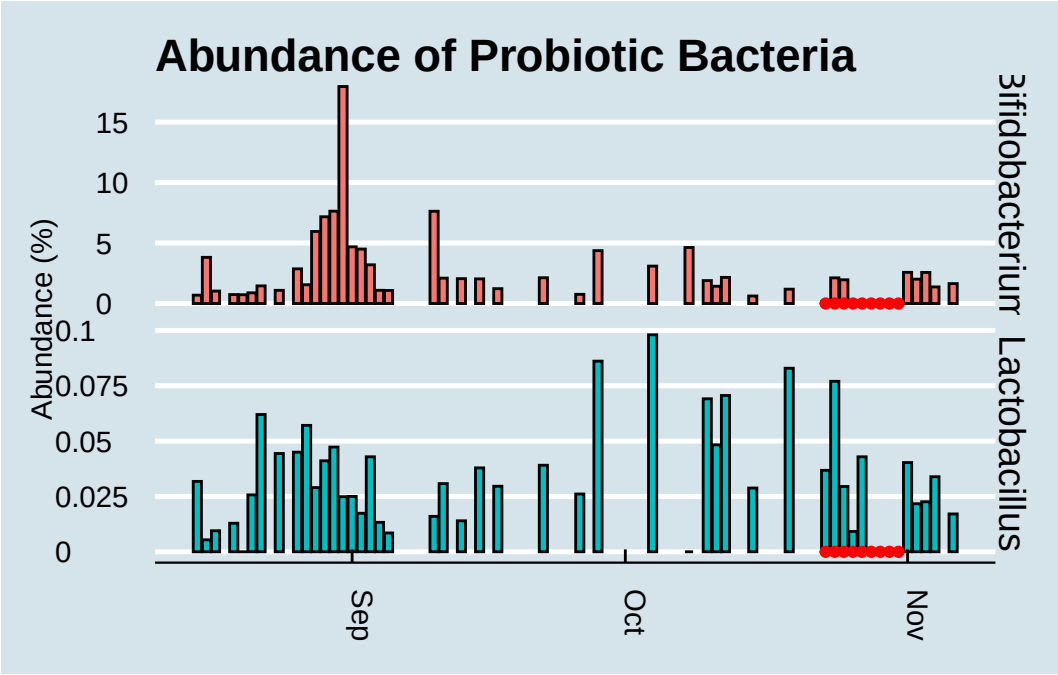


Figure 12.22: Percent abundance of key gut microbes over a three month period after taking a probiotic supplement.

Maybe my view of the microbe ecology, hoping to see results in only one or two genera, is too simplistic. We know that the gut is an ecosystem. If you add lots of one type of organism, maybe that affects the abundances and ratios of other microbes, all of whom are in constant competition with one another. Is there a way to tell *overall* how the microbes are changing?

Let's apply an *ordination* analysis. Essentially this means we look at all the samples together and work out how different the samples are from one another, based on some "distance metric" that compares the abundances of specific microbes. If the abundances of two samples are roughly the same, or if they tend to rise and fall together, then we plot them next to each other, and vice versa if they are not well-correlated. There is a mathematical way to do this where we combine all these different correlations over and over and pick just the two that seem to matter the absolute most, which we'll plot on a two-dimensional graph (Figure 12.23)

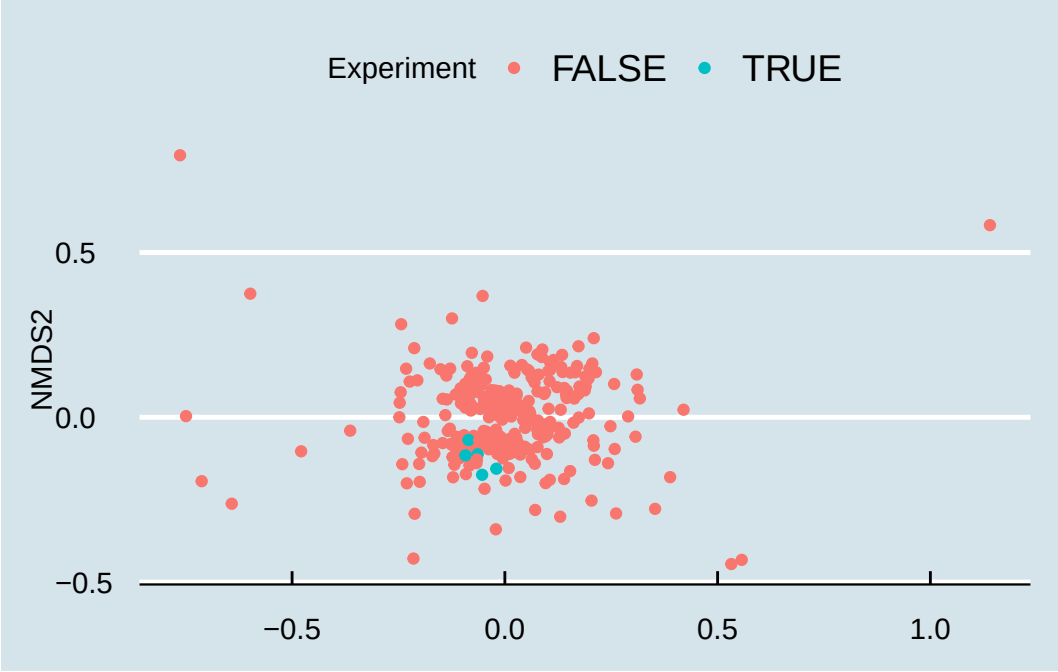


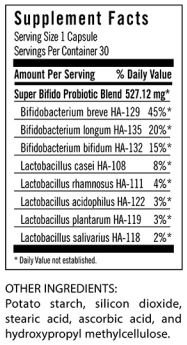
Figure 12.23: NMDS ordination (Bray-Curtis) of gut samples for ten months before and after taking probiotic supplements.

Hmm… that looks pretty random to me.

12.5.2.1 Other people

Since doing this experiment on myself, I've spoken with numerous others who've tried something similar: take a gut test, then start some type of probiotic supplement, and finally take another followup test a few days or weeks later.

Here's an example, "Jeremy", a healthy man in his 50s took this probiotic supplement: $42 for one month of pills:



Supplement Facts

Serving Size 1 Capsule
Servings Per Container 30

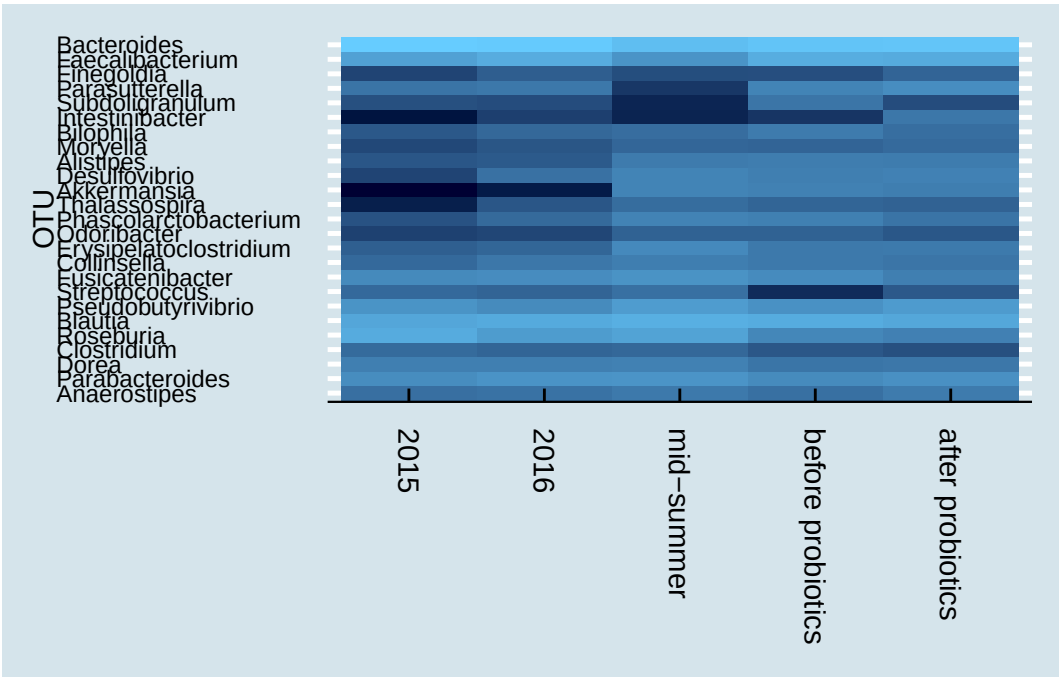
Amount Per Serving	% Daily Value
Super Bifido Probiotic Blend 527.12 mg*	
Bifidobacterium breve HA-129	45%*
Bifidobacterium longum HA-135	20%*
Bifidobacterium bifidum HA-132	15%*
Lactobacillus casei HA-108	8%*
Lactobacillus rhamnosus HA-111	4%*
Lactobacillus acidophilus HA-122	3%*
Lactobacillus plantarum HA-119	3%*
Lactobacillus salivarius HA-118	2%*

* Daily Value not established.

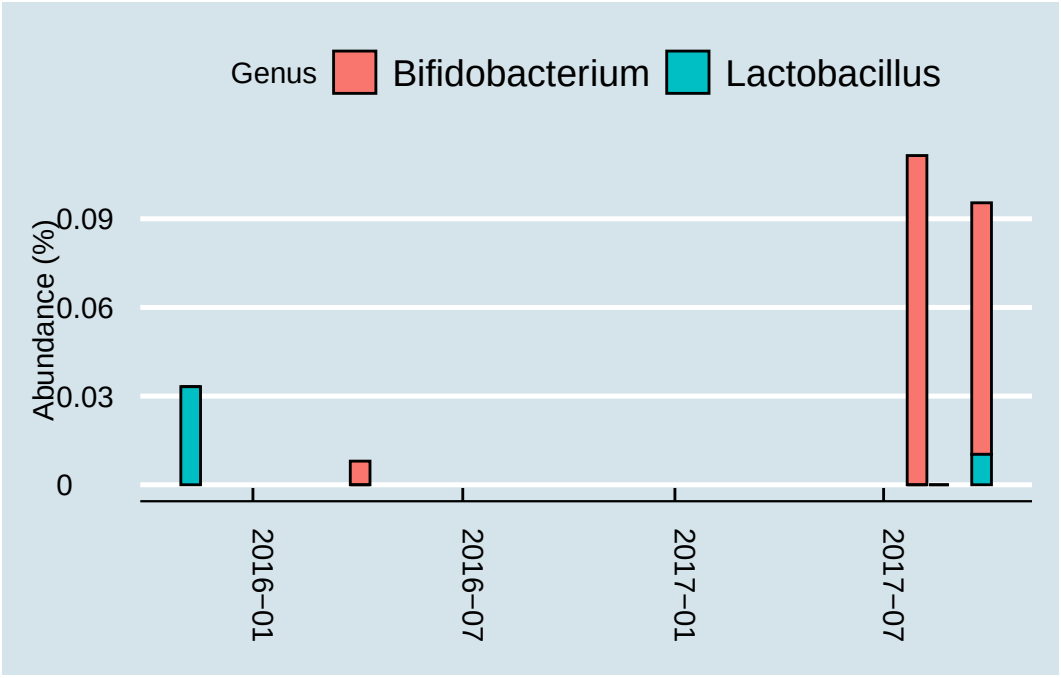
OTHER INGREDIENTS:
Potato starch, silicon dioxide, stearic acid, ascorbic acid, and hydroxypropyl methylcellulose.

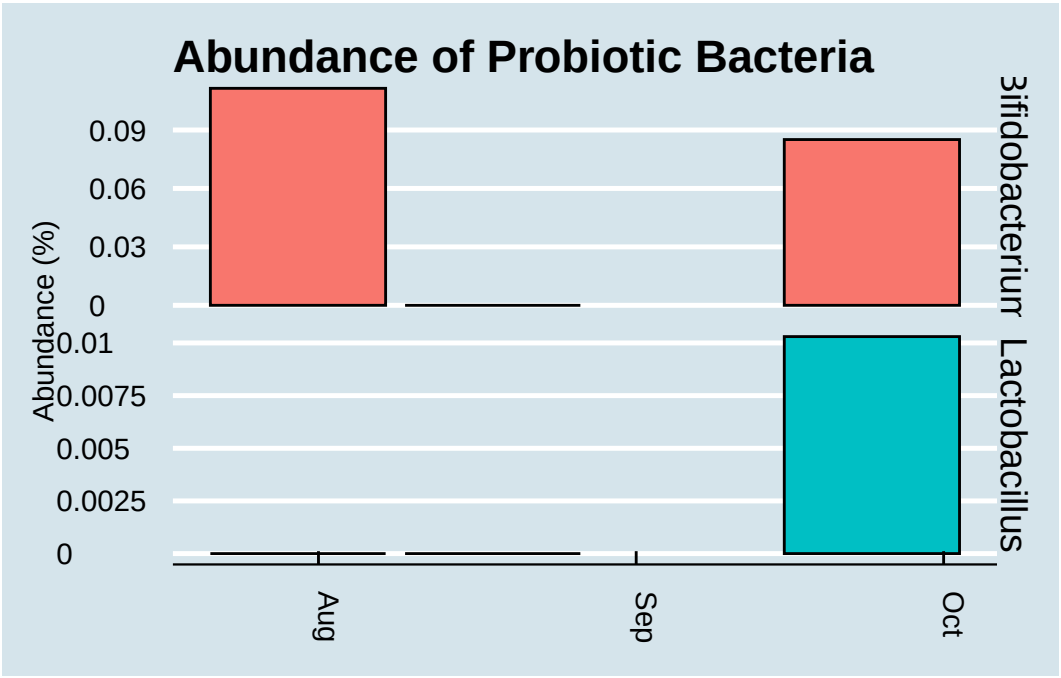
Figure 12.24: Super Bifido Plus Probiotics contains high amounts of live Bifidobacteria and Lactobacillus.

and here's the high level result:



Next let's look just at the microbes reported to be in the probiotic pills. Jeremy has three samples of interest: (1) taken in mid-summer, a month before starting the probiotics, (2) right before the month of pills, and (3) after completing 30 days of faithful pill taking.



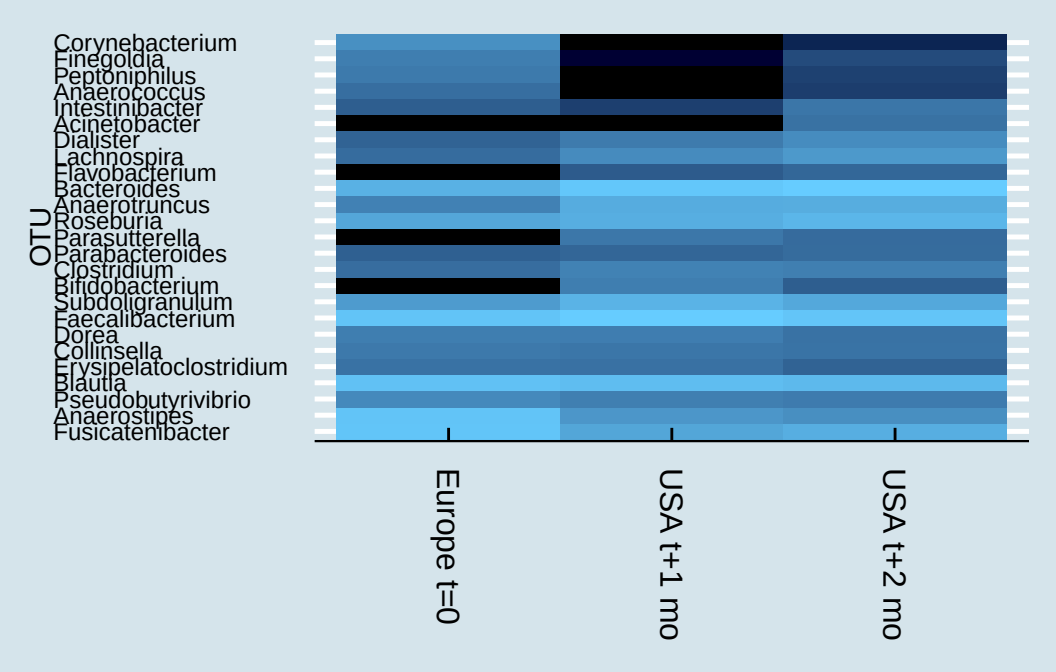


So although we *do* see a slight increase in both taxa, it's hard to pin it solely on the probiotics. After all, he was at even higher levels a month *before* starting the pills.

Also, looking more closely at the read counts, I see that the final sample had the lowest, about

36,000 reads versus the 80,000+ reads of the other samples. When dealing with low-abundance bacteria, this can matter, but it's impossible to tell precisely how much. The bottom line is that it's possible that the probiotics had no effect whatsoever, and even if there was an effect, it was probably quite slight.

In fact, probiotics appear to have less of an effect even than travel. Here's "Kevin", a European man who moved to the United States.



Notice how Kevin's microbiome shifted dramatically a month after arriving in the US. Soon after that, he began taking a probiotic supplement, but his gut – while different – hasn't shifted as much as it did from the international move.

12.5.3 VSL

The most tested probiotic is VSL#3, and recently a woman sent me her microbiome test results after taking Optibac for 4 days prior to her second test. (Figure 12.25).

In this case the abundances of these microbes went up significantly. Is that a coincidence? Hard to tell from a single sample, but perhaps this probiotic is one that makes it through and shows up in the results.

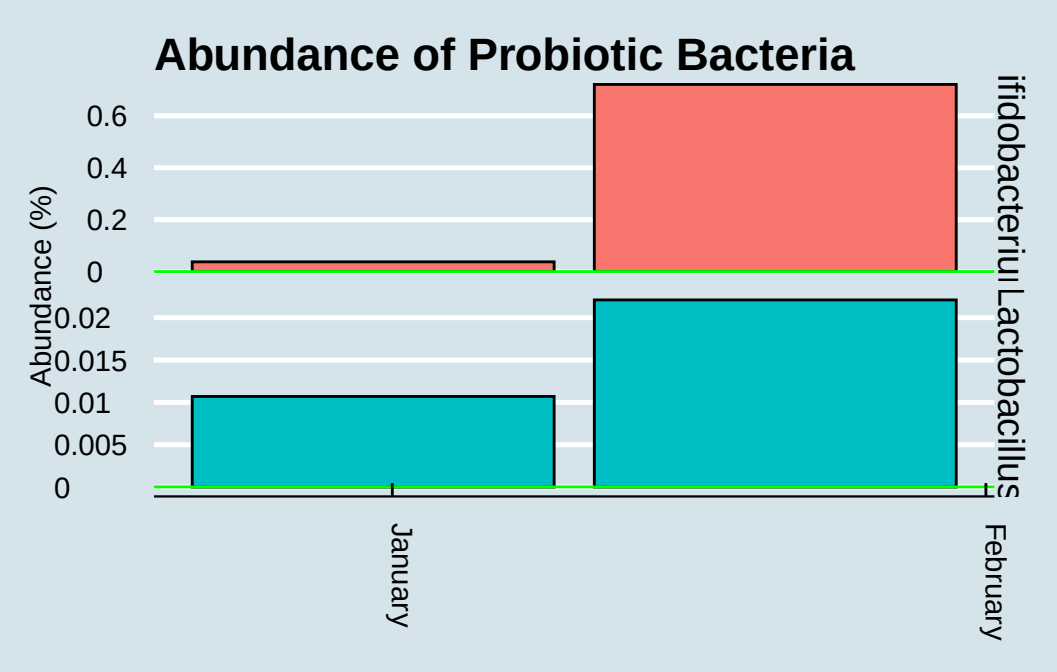


Figure 12.25: Change in key microbe levels after a course of VSL#3

12.5.3.1 (Tentative) Conclusions and next steps

It is very difficult to say with this analysis that the probiotics had any effect that is detectable by the uBiome Explorer test.

Further analysis required:

- *Consider other statistical analysis.* Although the two strains contained in the probiotic pill don't appear to cause a change in the gut microbiome results, are there other changes that can be detected statistically. Perhaps there are other taxa that show a significant change.
- *Other time horizons.* Maybe the changes don't happen immediately. Although at a high level, there doesn't appear to a noticable lag in the levels of the probiotic strains, perhaps a more sophisticated data transformation would uncover something.

12.6 Experiment: Gut Cleanse

Microbiome experiments are complicated by the difficulty of holding everything constant. Even if you are careful with precise amounts of the same food and exercise, you are still dealing with your existing microbiome with all its uncertainties, making it difficult to tell precisely

what caused a particular change. What if you could wipe the slate clean; start over with a completely new biome and just track *that,* along with precisely what you eat afterwards? What could you learn?

In this experiment, I tried exactly that, using a colon cleanse – the kind you do before a colonoscopy screening. By flushing all the bacteria from my system and carefully watching them grow back with day-to-day testing, I was able to get a better picture of the resilience of my microbiome.

The bottom line:

My gut microbiome recovers pretty quickly. Unlike antibiotics, which are known to cause long-term (and possibly permanent) changes, losing bacteria this way seems to matter only for a day or two. The missing microbes sprout right back just like a haircut. In two weeks it was as if nothing had happened.

Figure Figure 12.26 is a broad, phylum-level look at how the various microbes shifted in abundance. As you can see, all of these high-level colonies were back to the same proportions that had been before the cleanse.

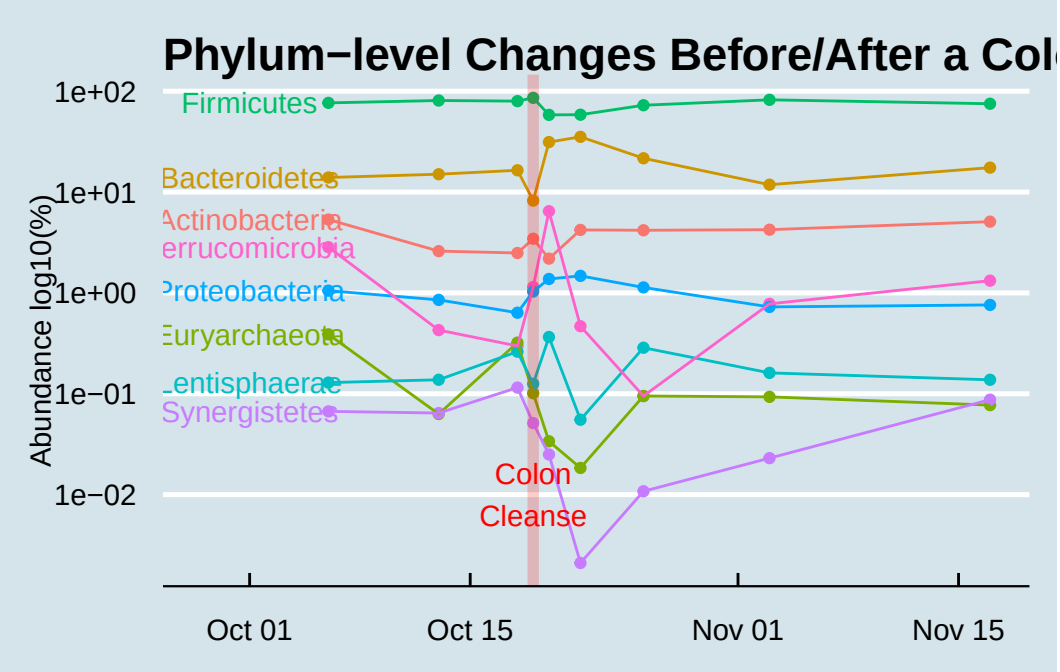


Figure 12.26: Overall phylum-level summary, from baseline (2 weeks before the cleanse) to CC (colon cleanse) to one month after CC.

Even at the more detailed, genus-level, whatever shuffling occured didn't look much different than the normal random variation I see in any month-long survey.

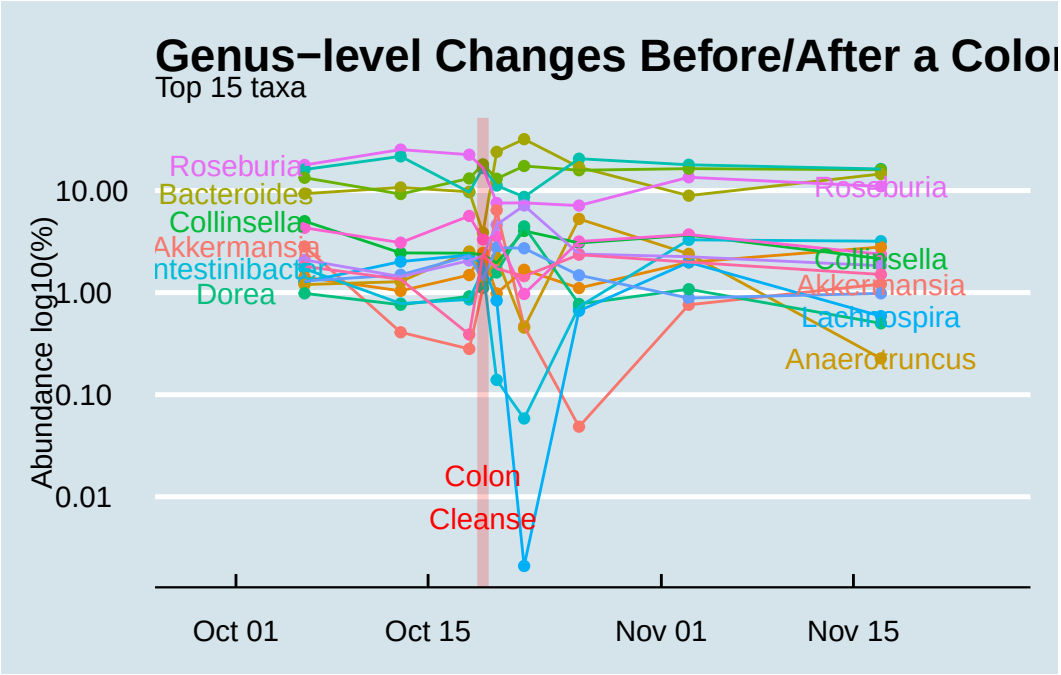


Figure 12.27: Overall Genus-level summary, from baseline (2 weeks before the cleanse) to CC (colon cleanse) to one month after CC.

Amounts and ratios changed, but not the specific organisms. Of course I lost a bunch of bacteria – that was the point – but surprisingly I didn't seem to gain anything really new, even after an aggressive attempt at re-seeding. I didn't gain or lose a single phyla. Other than amounts and ratios, I had to dig down to the Class level of the biological hierarchy to find anything that was permanently lost, and even at the very fine-grained Genus level, only two taxa that had been regularly present beforehand were now extinct. (*Holdemania* and *Methanomassiliicoccus*).

My overall gut diversity spiked the day of the cleanse and then plunged the following day, but soon it was right back to normal (Figure Figure 12.28)

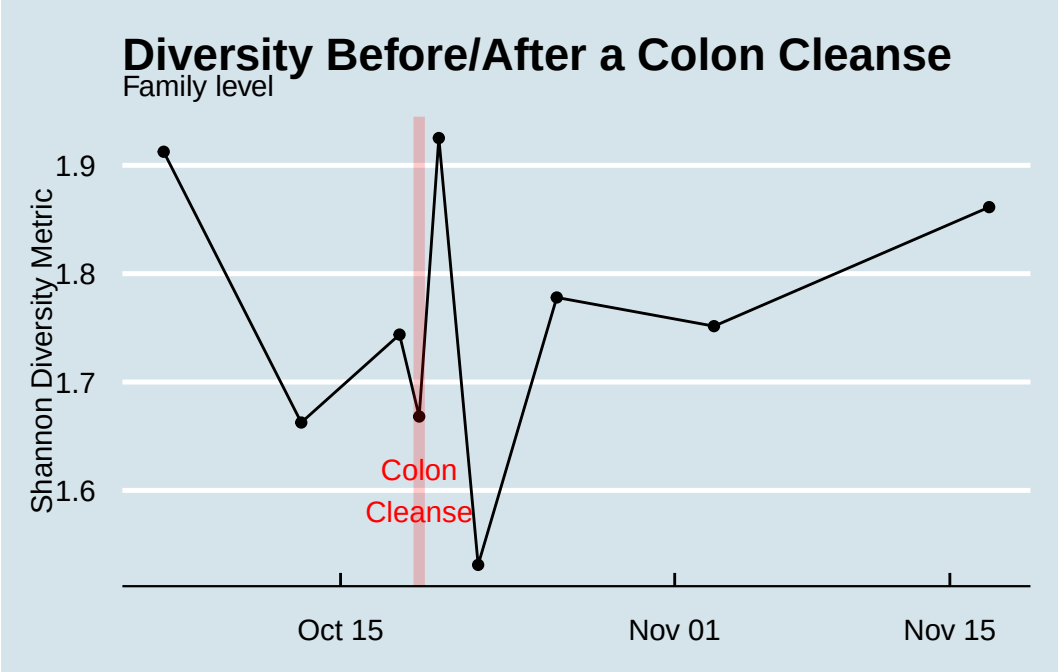


Figure 12.28: Shannon level diversity measures before and after the cleanse period.

A couple of weird microbes, at small amounts, made a brief appearance. I was intrigued by five new taxa that showed up just once, the day after the cleanse, and then disappeared. Maybe I found some that ordinarily get lost in the noise of the microbiome and only show up when the rest of the forest has been cleared. These are some hardy guys and I'm glad I know their names and can watch for them again: *Abiotrophia, Bacillus, Catonella, Christensenella, Parvimonas.*

It's pretty hard to make a significant change. These days a little googling will find plenty of web sites, books, diets, and supplements that claim to "fix" or "change" your microbiome. I'm a healthy, reasonably fit adult, so I'm not as motivated as somebody with a specific health problem, but I thought simply popping probiotics and eating a variety of new and fermented

foods would have a big effect. Nope. There are exceptions – new microbes will sprout when I drink homemade Kefir, or travel to China but it's much harder than you'd think.

Of course, I'm not the first to study microbiome changes after a colon cleanse. A 2015 European study found increases in *Dorea*, which interestingly I found as well (Figure Figure 12.29) .[13]

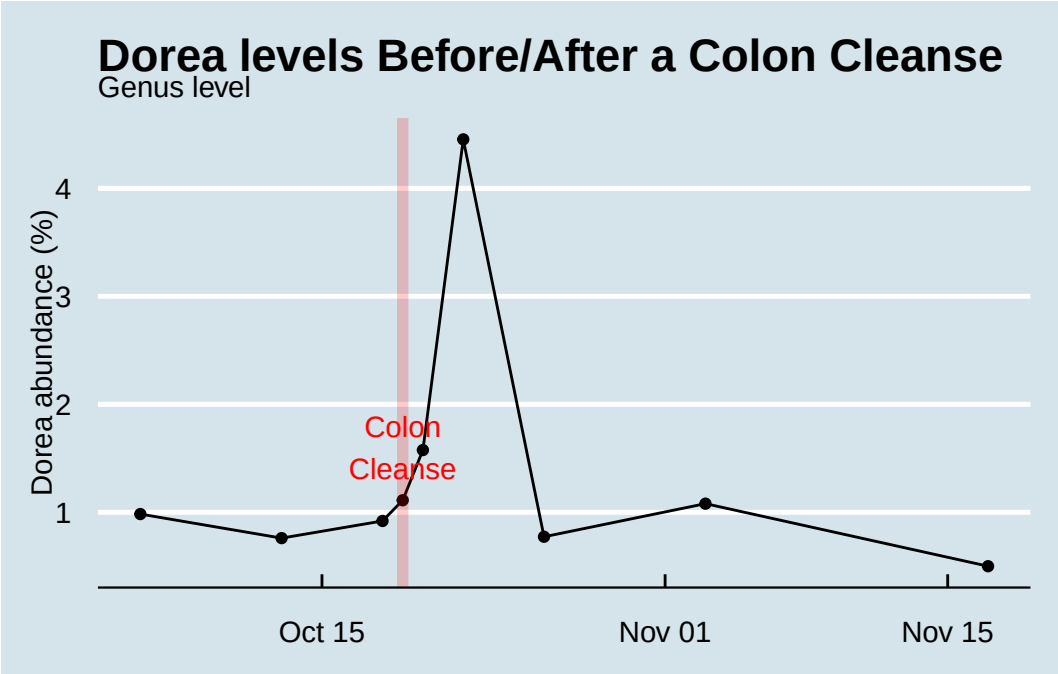


Figure 12.29: A rise in Dorea after a colon cleanse.

A more recent Japanese experiment Nagata et al. (2019) [14] also found, like me, almost no difference after two weeks. They also used a mass spectrometer to study the specific metabolites present in each sample, but again, after two weeks it's as though nothing had happened.

When I began this experiment I thought for sure I'd find something unusual and perhaps uncover a new way to modify the microbiome. Ultimately the main thing I learned is that the microbiome is incredibly robust. Even a complete reset won't change much.

[13] Read a detailed description of what microbes are noted in a bowel cleansing: Jalanka et al. (2015)
[14] Full text available here: https://www.nature.com/articles/s41598-019-40182-9.pdf

12.7 Hacking my sleep

Most people know about the hormone melatonin and that it has something to do with sleep. Some international travelers take it to counter the effects of jet lag, and some people take it regularly as a treatment for insomnia. You might vaguely remember that it has something to do with the pineal gland, a small organ tucked near your brain, but did you know that your gut contains *400 times*[15] more melatonin? Something like 80% of its precursors, as well as those of the similar mood-regulating neurotransmitter serotonin are made in the gut[16]

There are other reasons to suspect that sleep and the gut may be linked. Think of all those home remedies for insomnia: a glass of warm milk before bed, apple cider vinegar, non-caffeinated herbal teas – many of these are foods known to affect the microbiome.

A quick internet search for "gut microbe serotonin" will lead you to *Bifidobacterium infantis* which modulates tryptophan, the stuff in turkey that urban legends have long (and incorrectly) blamed for that sleepy feeling you get after Thanksgiving dinner. If you can raise the level of *B. infantis*, might it also improve sleep?

To understand how to grow these microbiobes, it helps to understand something about the bacterium itself. Fortunately, it's a well-studied organism, first identified back in 1899 as a common inhabitant of the intestines of breast-fed infants. Nowadays you can buy prebiotics that contain lots of bifido – or so they claim. Without rigorous lab independent verification of the claims, it can be hard to tell if the prebiotic form is helpful or not (and frankly, I'm skeptical)

Bifido is highly sensitive to oxygen, and flourishes best in environments like the colon that are anaerobic. It's also a strong fermenter of certain types of starches, called resistant starch, so-called because they resist digestion.

One of the best resistant starches is plain old potato starch, made by finely grinding tubers into a light, white powder. You can buy an organic version from *Bob's Red Mill* at most natural foods stores or high-end supermarkets. It's cheap, and tasteless, so it's often used in cooking, as a thickener for sauces.

[15] Chen (2011) or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3198018/
[16] Cryan and Dinan (2012) and see the excellent Blaser (2015) for more intriguing details.

Figure 12.30: No fiber in here.

The nutrition label on potato starch shows that it is essentially inert as a food. No calories, vitamins, or minerals, no fat, and not even any fiber. It's just zero on everything, because it passes right through the stomach. When cooked, it becomes a thick, gooey consistency that quickly is absorbed by powerful stomach acids, but if kept in its raw state, it slides right through into the colon.

Not many other foods make it this far undigested, so a rich unfermented wad of fresh potato starch is a real treat for the Bifido of the colon and they quickly begin to make the most of it, fermenting it into the precursors to tryptophan. At least, that's the theory.

Does it work? To find out, I started with two tablespoons the first day: just mix it in a glass of water (or other cold liquid[17] and drink it, preferably in the afternoon to give it plenty of time to make it to the colon and start feeding the microbes. On the second day I raised it to three tablespoons and kept it there for the following days. Anything larger might risk unpleasant gas or loose stools until my body adjusts. Within two nights it was obvious that something was working. I couldn't believe my excellent sleep!

After a few days, the sleep effect started to wear off, though I still felt much-improved. But could I trace the improvement to improved levels of Bifido? I continued to take potato starch randomly off and on for the next several months, measuring my sleep each night. What did the data say?

As you can see, there is almost no difference in the total sleep I enjoyed on nights following my eating a tablespoon or two of potato starch. I studied the data carefully, looking for possible ways the potato starch may have had an effect, but couldn't find proof that it worked[18]. It's worth noting that the sleep times (Z) in my data are calculated with a Zeo sleep tracking device which I wore strapped on my forehead to detect the subtle changes in electrical activity that come with sleep. Zeo let me calculate precise REM and Deep sleep numbers as well, but none of them seemed to be affected by potato starch.[19]

Unfortunately, when I ran this experiment I only received three microbiome results. The first came shortly after beginning to ingest large amounts of potato starch so I don't have a good "before" test. However, I *do* have one result taken after I had stopped the potato starch for several weeks. Both samples taken when consuming potato starch have much higher levels of *Bifidobacterium* than normal.

What *is* normal for me? Here's how I look during a typical three month period. (Figure 12.32)

Note that I have some *Bifidobacterium* in just about every sample (the red dots), but it doesn't look like there's a strong relationship with sleep. My daily average sleep (indicated in orange,

[17] you might try cocoa, which one study found significantly increases *Bifidobacterium* abundance: Tzounis et al. (2011) (full text)

[18] You can see the detailed code on my blog

[19] I tried correlating with other variables too, such as alcohol but found no effect. I *did* find a small effect during the days after taking Vitamin D supplements, but it barely met the bar for statistical significance. If there's an effect, it's not very strong.

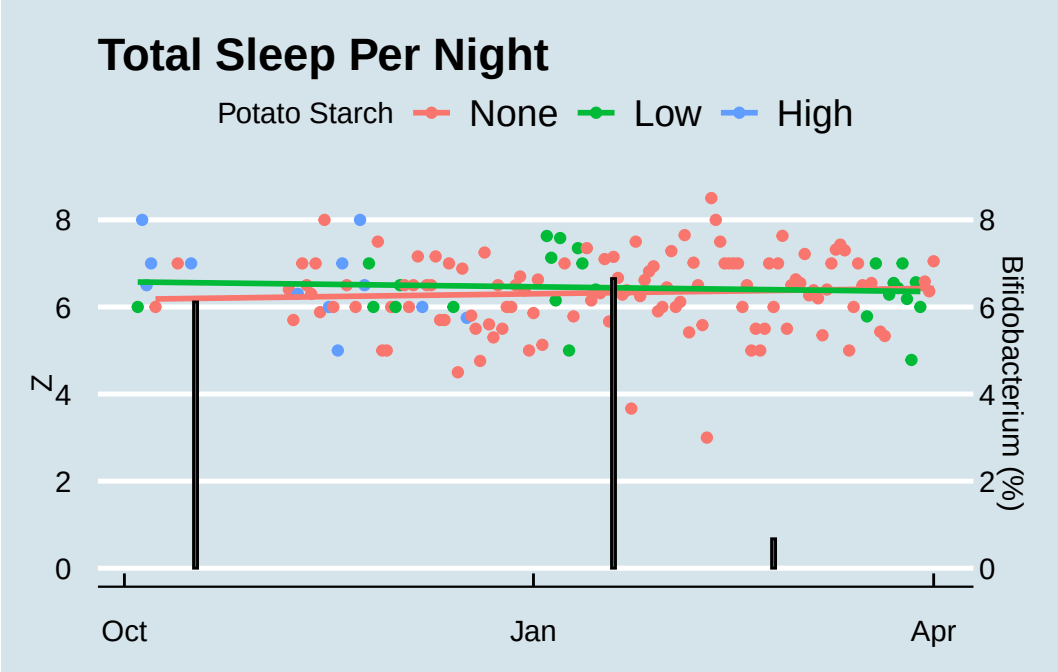


Figure 12.31: Potato Starch, Sleep, and Bifidobacterium levels.

averaged across the week) seems pretty constant, though the *Bifidobacterium* levels flucuate wildly.

Since describing my experiments, many people have contacted me to say they've tried the potato starch trick too with various levels of success. One person for whom it worked extremely well suggested from his own testing that the *amount* is critical. My 3-4 tablespoons per day was counterproductive, he said. The melatonin producers supposedly get swamped by that much food, so it's better to give them a much tinier amount.

Unless you test daily, it's hard to see subtle patterns in microbiome samples, and my original experiments weren't frequent enough to tell why (or whether) the *Bifidobacterium* is changing. So, taking my friend's advice, I tried some smaller amounts of potato starch. How do those look?

Here it's more obvious that any potato starch had little to do with the rise and fall of my gut *Bifidobacteria*. So why were the percentages so much smaller in this experiment than in the higher 6+% numbers I found while doing my original, more rigorous sleep measuring test above? Maybe it's the tinier amounts? We'll have to test again to understand for sure.

An interdisciplinary team of scientists says resistant starch can change the ratio of Firmicutes to Bacteroidetes[20]. I calculated the ratio for my own testing and found some interesting, often

[20]see Maier et al. (2017) full text

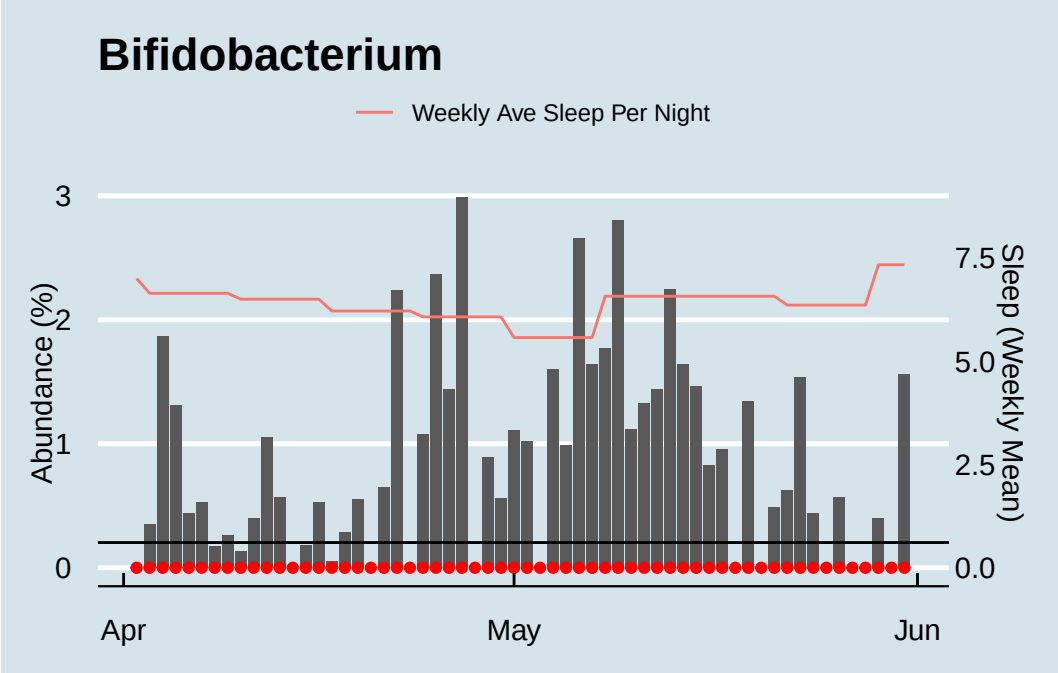


Figure 12.32: Bifido abundance over time. Red dots are days for which there is a sample. Blue line is the medium value for healthy people. Orange is the average sleep per night for each week.

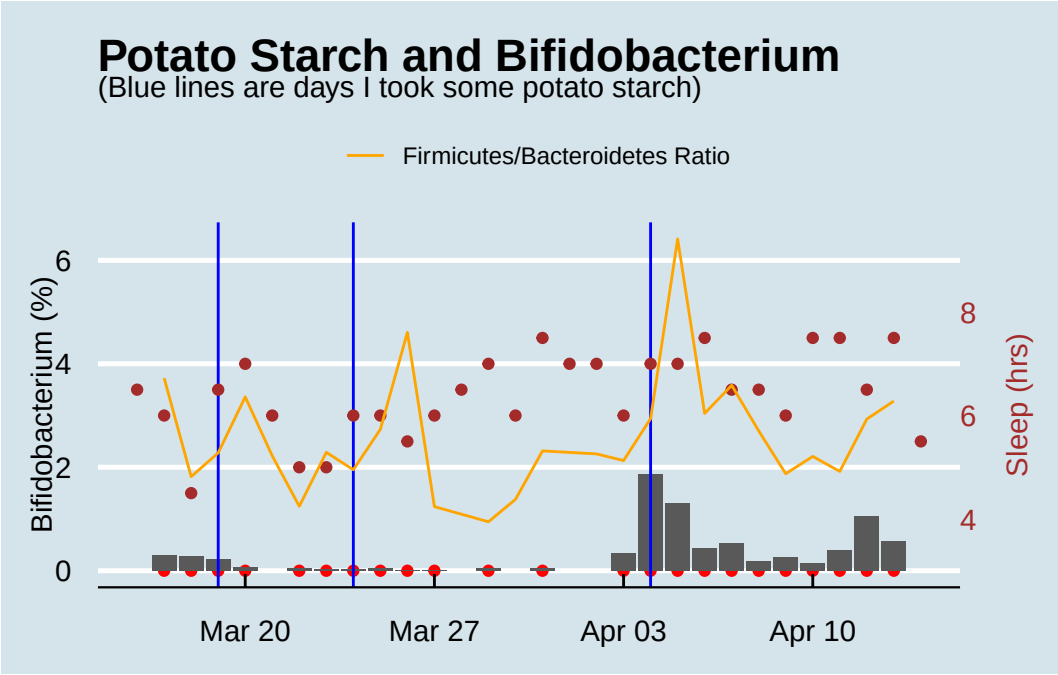


Figure 12.33: No apparent relationship between small amounts (1-2 tsp) of raw potato starch and Bifidobacterium abundance

dramatic rises a day or two after taking the potato starch. (Figure 12.33)

Conclusion: There's a possibility that, in high enough amounts, potato starch increases my *Bifidobacterium* levels. Whether it increased microbes associated with melatonin production is less clear, but it's hard to show that the potato starch caused a noticable change in my sleep.

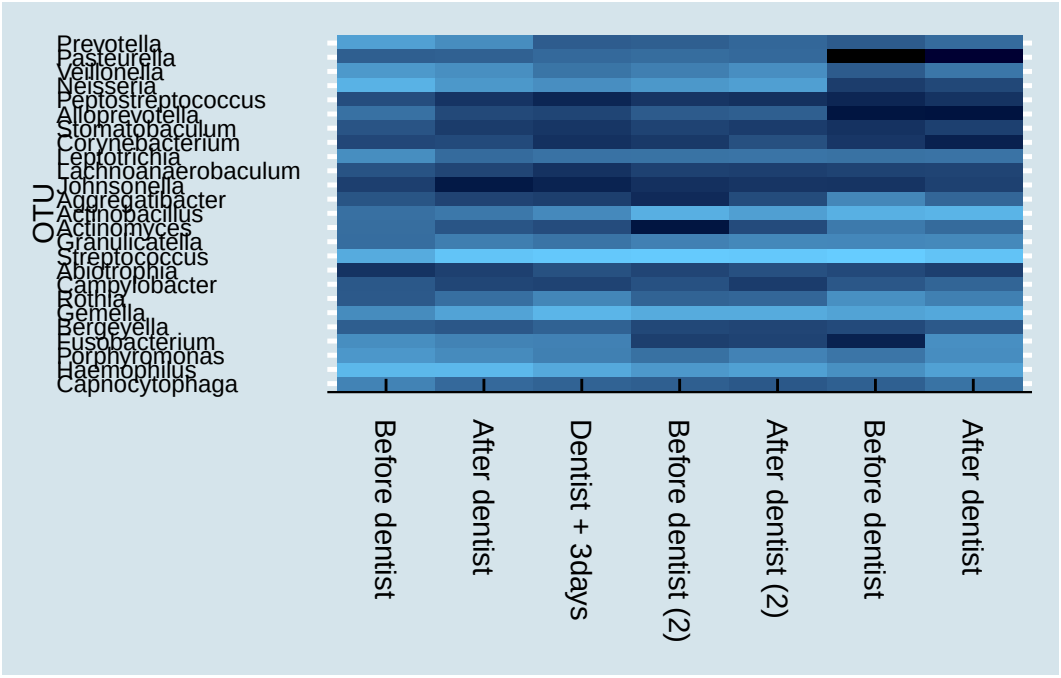
12.8 Visiting the dentist

The gut biome is interesting enough, but bacteria colonize just about every part of the body, so here's an experiment to measure the mouth bacteria and how the varieties shift after a visit to the dentist.

The mouth harbors its own unique ecology of bacteria, much of which is entirely unexplored. Scientists from the Forsyth Institute in Boston are at the cutting edge of the research, and have characterized many of the species found in their test subjects mouths, but widespread human trials are still years from producing the kinds of results we see from the gut biome research.

So far the research *is* clear that many cavities are associated with one nasty species: *Streptococcus Mutans*. This bug contains receptors that adhere to the surface of the tooth, creating a slimy biofilm where, under the right conditions they breed rapidly. Interestingly, just a few hundred bacterial cells is enough for it to begin its work, feeding on glucose to create a reaction that combines with the tooth enamel to form plaque. The ever-present lactic acid in the mouth, a critical component of pre-digestion, reacts with the plaque to remove calcium from the tooth, leaving small, ever deepening holes that will destroy the tooth unless the dentist intervenes with a filling.

I visit my dentist in April and October each year, and I measure my mouth biome before and after each April visit. Unfortunately I don't have before/after for the October samples, but I *do* have a sample taken a few days *afterwards*. Let's look at an overall heatmap picture of all the dentist-related mouth samples:



These are five samples taken over the course of one year, when many things can change. Nevertheless, the differences between the before/after samples is strikingly obvious. Despite being sampled only a day apart, there is clearly a major shift in the mouth microbiome after a dentist appointment and cleaning.

Here's a more numerical breakdown at the genus level of the top ten microbes and their abundances before and after first visit:

	% before	% after
Haemophilus	22.3237	23.0557
Neisseria	17.9975	6.2514
Streptococcus	13.4156	36.0979
Prevotella	8.8309	3.9503
Veillonella	6.5300	4.3380
Porphyromonas	6.1201	3.6216
Leptotrichia	4.1791	0.9552
Fusobacterium	4.0991	2.5904
Gemella	3.8491	9.7999
Capnocytophaga	2.6722	0.8850

The most abundant taxa, *Haemophilus*, stays relatively stable, while abundances of the second two taxa *Streptococcus* and *Neissaria* seem to switch places. The other taxa in the top ten also seem to drop in abundance, except for *Streptococcus* and *Gamella*.

Anything in the genus *Capnocytophaga* is an opportunistic pathogen, so I say good riddance. Usually they're fine, but if your immune system dips they can turn bad.

To understand more precisely what changed, let's look more closely just at the ones that disappeared:

	% before
Centipeda	0.0614
Chryseobacterium	0.0385
Bilophila	0.0057
Bacteroides	0.0042
Dialister	0.0028
Akkermansia	0.0028
Blautia	0.0028
Stenotrophomonas	0.0028
Mycobacterium	0.0028
Delftia	0.0028

All of these unique microbes are of such tiny abundances that it's hard to rule out simple contamination or other problems with the sampling. Still, it is interesting that there was nothing new (at the genus level) in the "after" sample that wasn't in the "before". This is consistent with the expectation that a dental cleaning would, if anything, tend to *remove* taxa rather than introduce any new ones.

But that was just for a single dental visit. What happened when I repeated the experiment the following year?

	% before	% after
Streptococcus	45.8502	43.1782
Actinobacillus	15.7059	8.5416
Gemella	13.3460	13.3400
Haemophilus	6.4258	8.7729
Neisseria	6.2026	8.4911
Granulicatella	2.3758	3.2374
Veillonella	2.2163	4.2491
Leptotrichia	1.3154	1.3874
Porphyromonas	1.2118	2.5798
Pasteurella	1.0204	0.8744

Interestingly, this time my most abundant taxa is *Streptococcus*, instead of *Haemophilus*.

Like last time and as expected, I found no new taxa *after* the cleaning, but here are the genus-level items that disappeared, all at such low abundances that we should probably chalk them up to contamination or other errors that creep in unavoidably between the time I take the sample and when they show up in my results.

	% before
Moryella	0.0558
Stenotrophomonas	0.0318
Mycobacterium	0.0239
Centipeda	0.0239
Candidatus Saccharimonas	0.0159

Let's start with the genus level. How much *Streptococcus* has been in my mouth, and to the degree that we know at the species level, which types of species are there?

Hmm, lots of different species here. But what about the cavity-linked *S. Mutans*? It turns out that I *do* have a tiny bit, but in just one sample long ago. And sure enough, my dentist confirms that I have no cavities.

Keeping *S. Mutans* at bay is an important way that I'll try to avoid cavities, so to continue the experiment, I'll look at what I can do to manipulate the mouth biome, beyond what I eat and drink. A key part of that is how I brush my teeth.

Like most Americans, for years I brushed exclusively with one of the name brand toothpastes, usually Crest or Colgate. But looking more closely at the labels, I see two ingredients that will be of interest to my oral microbiome: triclosan[21] and sodium lauryl sulfate, both of which are are known to affect microbes. In addition, the fluoride in the paste works partly by making the tooth enamel more difficult for bacteria.

Pre-modern humans didn't have toothpaste, and certainly not the antimicrobial kinds that have become popular only in the past generation. Of course, tooth decay was a painful reality for many of our ancestors as well, but there is good evidence that serious teeth problems didn't begin until the widespread availability of sugar after the European immigration to America five hundred years ago. Skulls of humans before agriculture show almost no tooth decay. Wild animals, including primates like gorillas and chimpanzees get far fewer tooth problems than modern people, another clue that teeth brushing isn't the whole story.

Could it be that a healthy mouth requires a healthy *diversity* of bacteria, including versions of *Stroptococcus* that out-compete the cavity-causing kinds? But toothpaste with triclosan and other anti-microbials are wide-spectrum: they don't target just the "bad" cavity-causing organisms. They also kill other species needed for digestion, or to control bad breath.

To find out more about whether oral diversity is a good or bad thing, for my continuing mouth experiment I changed toothpaste. Rather than continue with one of the leading fluoride brands, I switched to a more "natural" brand whose ingredient list does *not* contain antimicrobials.

[21] Note that Crest hasn't used this in its products since 2014

12.9 Sniffles

I don't often get colds, at least not *serious* ones that keep me in bed. But this past Spring, there had been something going around. Most of my family was spared, but then my teenager had to stay home from school. A few days later I felt a prickly sensation in the back of my throat. Nothing serious, but just enough to make me wonder if I might be catching one too. I started to drink more liquids, tried to slow down at work, took extra care to get to bed on time, and did whatever else I could to stave it off.

To no avail. Here's how my nose microbiome faired a week before, the week of, and the week after a nasty rhinovirus hit me with the sniffles and a cough Figure 12.34.

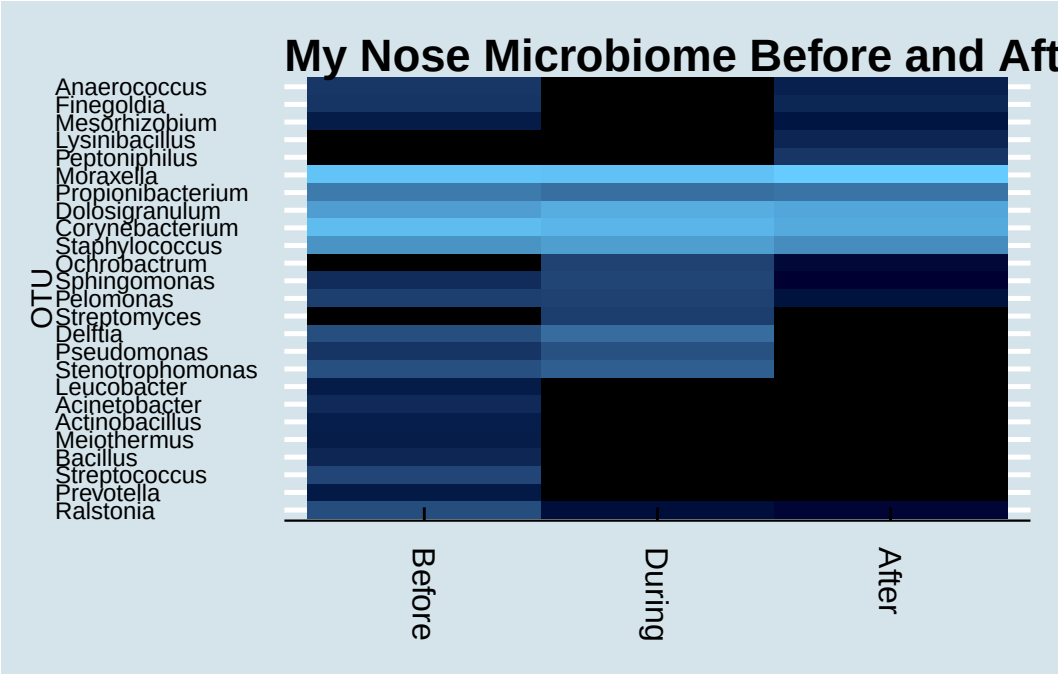


Figure 12.34: My genus level nose microbiome as tested the week before, during, and after a case of the sniffles. Lighter colors mean higher abundances.

When I want to study an unfamiliar microbiome, generating a heat map like this is often the first step toward finding something unusual. In this case we see a notable increase in levels of *Ochrobactrum*. Is that a coincidence?

To find out, I first generated a few more heatmaps, covering a longer period of time. Although I think of myself as relatively immune from colds – at least bad ones – when I looked at my notes I remembered that there had been a similar, mild bout of the sniffles back in December. I looked more broadly at each of the taxa that seemed to rise and fall throughout that period

and one by one I eliminated various culprits that might be associated with my colds. Except this one.

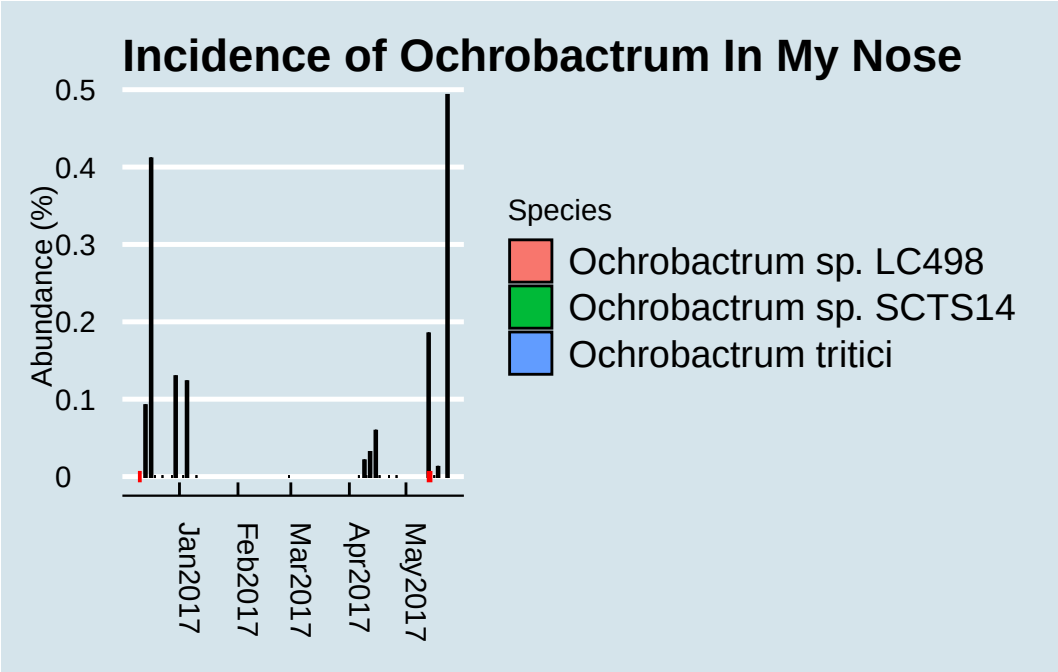


Figure 12.35: Red line are dates when I had the sniffles.

The red dots are days when I had a noticable bout of sniffles. It is highly intriguing that, after years of nose microbiome testing, the only flareups of *Ochrobactrum* happen in the week or two after catching a cold. I've looked at my other samples, going back to 2014 and these are the only peaks of this taxa.

What about the other rise that happened during April? It wasn't associated with a full-blown cold, at least not one that I noticed, but I *was* traveling that week, with several hours spent on airplanes and in unfamiliar hotels. Oddly I didn't see a similar increase during other trips. Is it possible that I actually *did* catch a cold of some kind, but I just didn't notice it?

Now, I'm just a single data point, so data of this kind hardly proves anything. But like all personal science, it gives us some new, possible questions to ask, some lines of inquiry that might be useful for professional scientists to consider.

But before getting too excited, what do we know about this bacterium? It's more commonly found in plants than in people, especially the area around plant roots called the rhizosphere.[22] It's not unknown in humans, though. A few Google searches reveal that this genus includes

[22]http://onlinelibrary.wiley.com/doi/10.1111/j.1462-2920.2005.00891.x/full

a rare pathogen, *O. anthropi*, that is sometimes seen in immunocompromised people.[23] In my nose, uBiome's bioinfomatics pipeline labeled most of my new species as *O. tritici*, which was identified last year as a pathogen [24] infecting a 70-year-old man infected with jaundice.

I think a better question is what a bacterium would have to do with the common cold, which as we know is caused by a virus – a completely different kind of microbe and one that is not detectable by the 16S rRNA technology used in my microbiome testing. I can only speculate, but I wonder if maybe the cold was actually caused by a *phage*, a virus that infects not human cells but bacteria. Is it possible that a phage, by killing off or otherwise modifying some of the "normal" bacteria in my nose, might allow a different bacterial species to have a brief runup in abundance?

Finally, the really cool discoveries relate to treatment. What if we could find a microbe, a 16S-recognizable one that appears *before* coming down with the sniffles? Just predicting when I'll get a cold is useful, even if I can't stop it. Of course, even better would be a discovery of some microbe that could out-compete or otherwise destroy the one associated with the cold virus. So far I haven't found a candidate bacterium that is clearly associated with the *onset* of a cold, but from now on I'll be much more careful when I see *Ochrobactrum*.

12.10 Other foods and my microbiome

Food affects the microbiome, but can we tell more about which types of food and the specific microbes they affect?

I carefully tracked precisely which foods I ate and computed the totals for the main macronutrients. How do the abundance levels of a typical microbe change in response?

Hard to see any particular patterns, but keep in mind that some foods take longer to digest than others. The abundance levels might be difficult to spot unless we did a carefully controlled experiment involving only a single type of food.

Instead of the macronutrients, maybe we can learn something by looking at a specific food. Flax is often described as a powerful food source for gut bacteria. Can we tell which microbes are most affected, and by how much?

By tracking daily, I'm able to see trends and relationships that wouldn't show up in a normal large trial.

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[23] http://jcm.asm.org/content/51/4/1330.full
[24] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4773274/

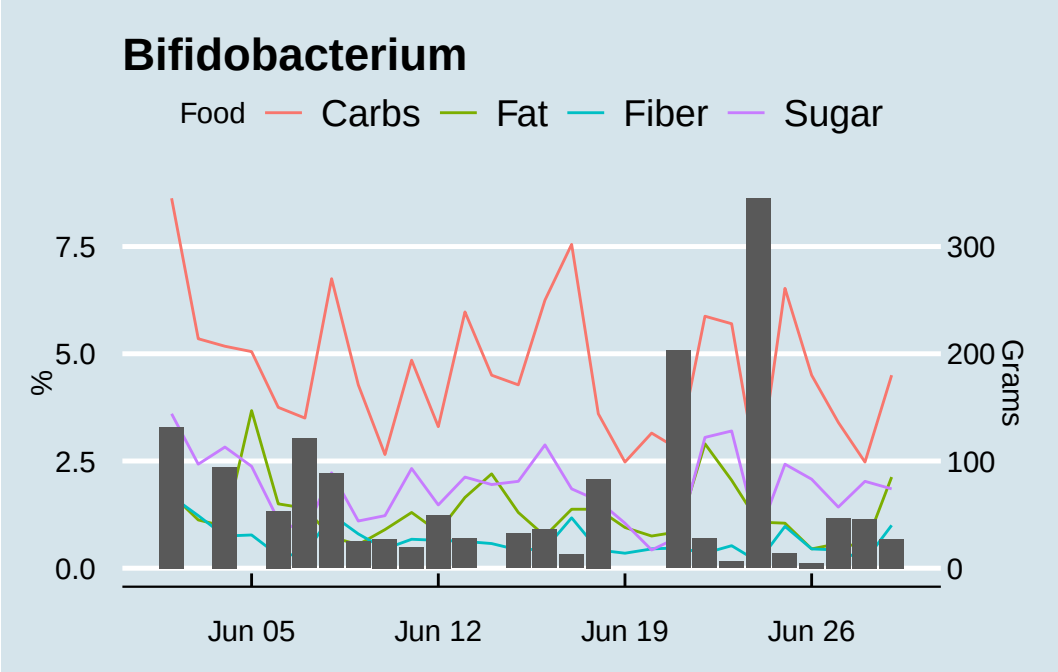


Figure 12.36: See the interactive version of this chart: https://personalscience.shinyapps.io/shinyactinodb/

I occasionally eat ground flax seed, a few tablespoons mixed into other foods. How does it affect my microbiome compared to the days when I *don't* eat it?

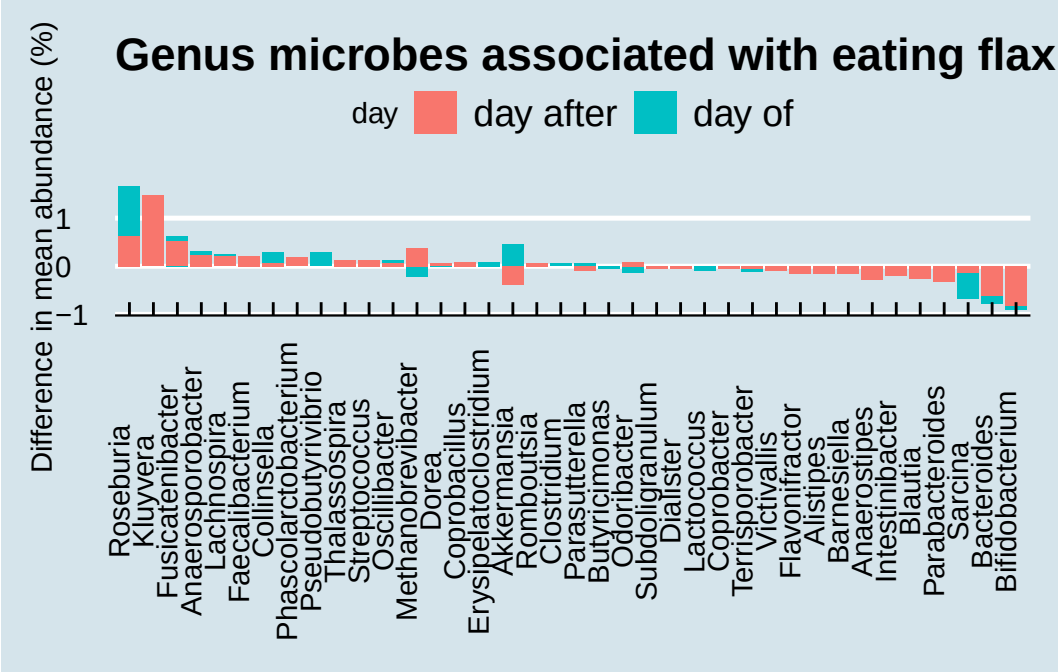
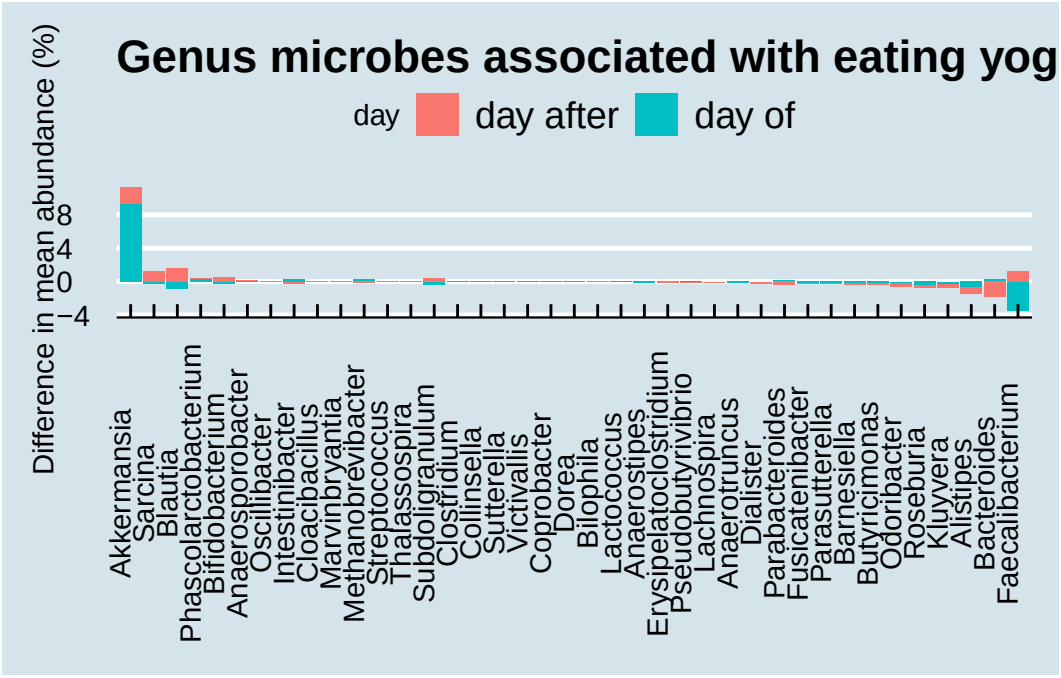
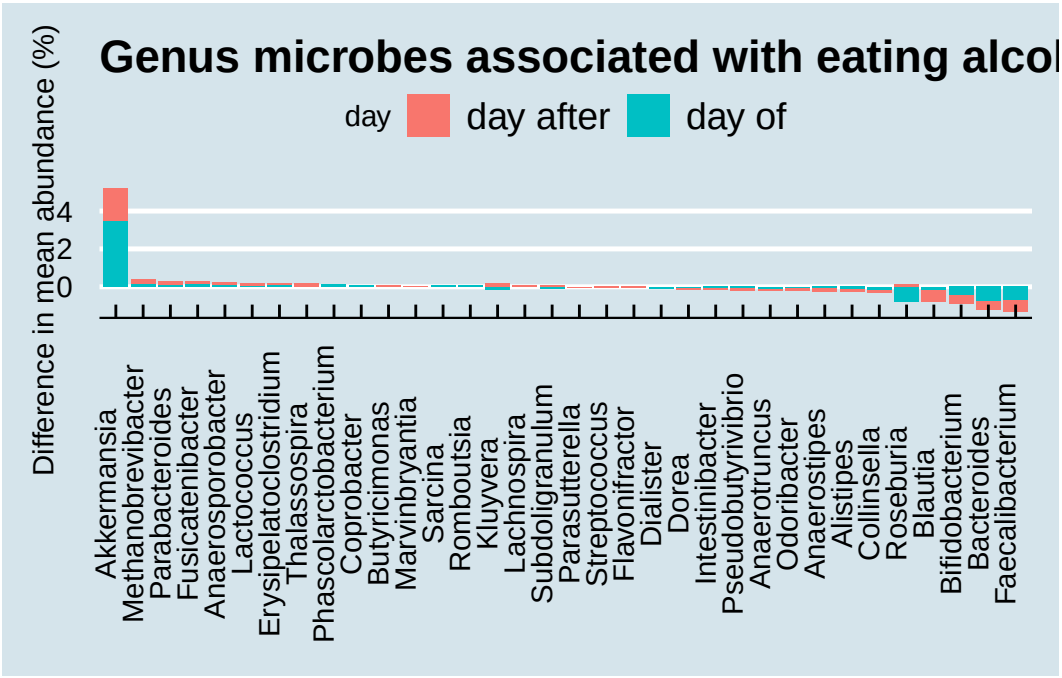


Figure 12.37: Red dots mark days when I add flax.

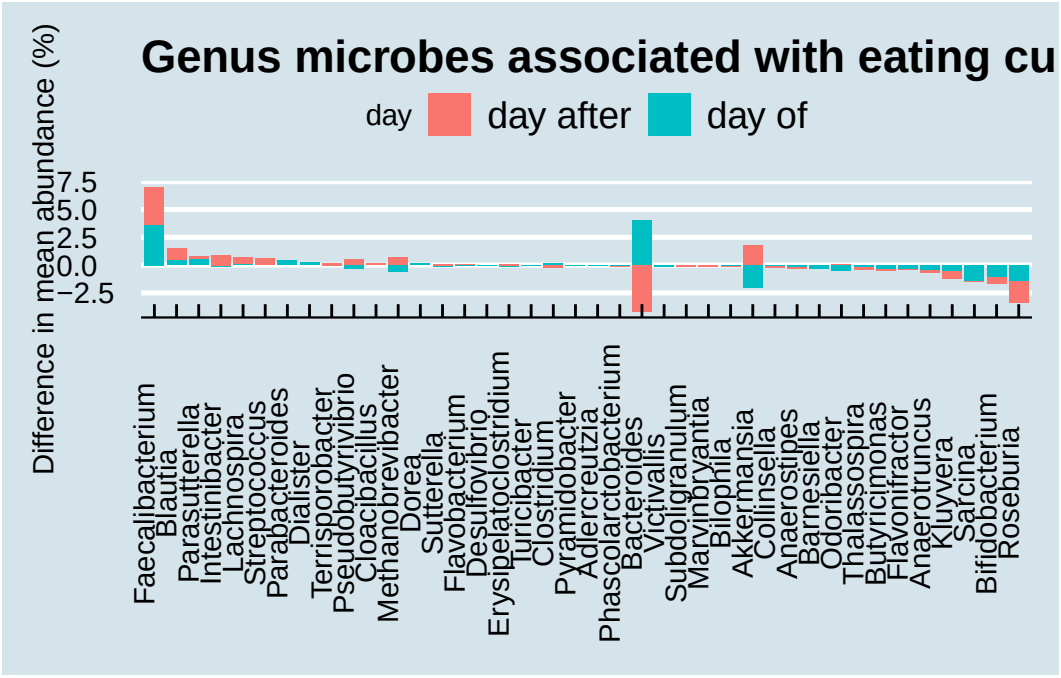
Notice how in nearly every case, flax-eating appears to affect microbiome abundances on both the day of the sample *as well as* the day following. This makes intuitive sense: if flax has an effect, you'd expect it to linger for a day or two. If there were no effect, you might expect the levels to come and go randomly.

what about other foods? How about yogurt?


Genus microbes associated with eating yog
day
day after
day of
Difference in mean abundance (%)
8
4
0
−4
Akkermansia
Sarcina
Blautia
Phascolarctobacterium
Bifidobacterium
Anaerosporobacter
Oscillibacter
Intestinibacter
Cloacibacillus
Marvinbryantia
Methanobrevibacter
Streptococcus
Thalassospira
Subdoligranulum
Clostridium
Collinsella
Sutterella
Victivallis
Coprobacter
Dorea
Bilophila
Lactococcus
Anaerostipes
Erysipelatoclostridium
Pseudobutyrivibrio
Lachnospira
Anaerotruncus
Dialister
Parabacteroides
Fusicatenibacter
Parasutterella
Barnesiella
Butyricimonas
Odoribacter
Roseburia
Kluyvera
Alistipes
Bacteroides
Faecalibacterium


Genus microbes associated with eating alcol
day
day after
day of
Difference in mean abundance (%)
4
2
0
Akkermansia
Methanobrevibacter
Parabacteroides
Fusicatenibacter
Anaerosporobacter
Lactococcus
Erysipelatoclostridium
Thalassospira
Phascolarctobacterium
Coprobacter
Butyricimonas
Marvinbryantia
Sarcina
Romboutsia
Kluyvera
Lachnospira
Subdoligranulum
Parasutterella
Streptococcus
Flavonifractor
Dialister
Dorea
Intestinibacter
Pseudobutyrivibrio
Anaerotruncus
Odoribacter
Anaerostipes
Alistipes
Collinsella
Roseburia
Blautia
Bifidobacterium
Bacteroides
Faecalibacterium

Or cupcakes:



12.11 Collection methods

To understand a result, we need to know more about the overall variability of the microbiome. As we showed previously, the day-to-day changes can be substantial, but what about variability in the sample itself? To find out, I tried sampling the same, uh, evacuation in several different ways. (Warning: some of the discussion here may get a little precise).

I submitted two swabs to the lab. One of them I simply poked somewhere in the middle of the sample. In the other, I placed the entire sample into a plastic bag and gently "blended" it as best I could, rolling and kneading it back and forth until it was as mixed as I had patience to try. Here are the results at the genus level:

	Middle	Blended
Roseburia	19.80	17.88
Blautia	12.45	5.87
Bacteroides	9.96	14.08
Faecalibacterium	6.33	15.93
Sarcina	4.41	3.13
Intestinibacter	3.51	0.55
Collinsella	2.33	1.60
Alistipes	1.84	2.82
Kluyvera	1.83	2.63
Odoribacter	0.99	2.72

As we can see, there is a significant difference. If you are merely swabbing from a single wipe, it'll clearly matter a great deal that you wipe consistently from sample to sample.

Sometimes I'll have more than one chunk in the same "session". Here's what happens when I wipe from the first chunk and then the second:

	First	Second
Akkermansia	32.51	9.11
Bacteroides	11.85	6.94
Faecalibacterium	9.27	15.52
Sarcina	5.07	7.14
Roseburia	4.50	7.01
Blautia	4.48	7.36
Parabacteroides	2.70	1.69
Bifidobacterium	2.11	5.49
Methanobrevibacter	1.79	5.10
Alistipes	1.68	2.17

But what if I sample twice on the same day? Although I tend to be a once-a-day guy, there are occasions when I'll need to go more than once and I have a few examples with data from the same day.

	Day 1 Morning	Day 1 Afternoon
Bacteroides	16.51	15.00
Blautia	8.29	6.73
Roseburia	7.08	7.00
Akkermansia	6.93	8.43
Sarcina	5.83	4.17
Faecalibacterium	2.70	20.16
Alistipes	2.54	2.54
Parabacteroides	1.39	1.37
Fusicatenibacter	1.00	1.68
Methanobrevibacter	0.09	3.60

	Day 2 Morning	Day 2 Afternoon
Faecalibacterium	21.51	19.55
Sarcina	8.77	5.74
Bacteroides	8.73	17.59
Roseburia	8.41	5.64
Blautia	8.32	6.32
Akkermansia	3.51	4.89
Pseudobutyrivibrio	2.84	2.40
Anaerostipes	2.82	1.74
Subdoligranulum	2.64	2.36
Methanobrevibacter	1.81	1.69

	Day 3 Morning	Day 3 Afternoon
Faecalibacterium	21.88	24.14
Bacteroides	13.17	16.84
Roseburia	9.67	7.46
Blautia	8.67	7.45
Bifidobacterium	7.49	5.03
Sarcina	3.12	1.96
Subdoligranulum	3.02	2.79
Lachnospira	2.03	2.31
Fusicatenibacter	1.83	2.12
Akkermansia	1.36	1.76

Or maybe it's just me. Something odd about my own microbiome, perhaps, or just something related to how I sample?

Here's another sample somebody sent me, two swabs from the wipe:

	Sameday1	Sameday 2
Roseburia	32.46	27.09
Faecalibacterium	9.21	10.22
Bacteroides	8.02	9.62
Blautia	6.30	6.68
Sarcina	4.41	4.35
Collinsella	1.95	1.89
Pseudobutyrivibrio	1.93	1.68
Anaerostipes	1.70	1.72
Barnesiella	1.69	1.94
Thalassospira	1.38	1.92

Person 2: separate swabs from the same sample

Same day, very different results. My conclusion is that to ensure results are comparable, you must be extra careful to adopt similar methods across samples. My advice is to never rely on a single test; always get more than one sample, and don't make conclusions unless you've seen multiple results, often over several days. And when you *do* take a sample, try to move the swab through as much of the DNA as possible. It's not as convenient, but unfortunately this means taking a chunk (not a wipe) and swabbing throughout. Generally, the more DNA you can collect, the better.

Ultimately the real lesson is to be humble about what we can learn from a single sample while simultaneously noting that there *is* a signal in the noise. After hundreds of samples, I see variance, but not *too* much variance. There really is a distinct signal in my microbiome, one unique to me, and worth uncovering.

Diversity	Location
2.817829	Blended
2.777073	Middle

Diversity differences between two swabs taken from the same sample.

Scientists at uBiome released results from experiments testing the variability of gut samples. My own experiments show considerable day-to-day variability, so I was interested to see their conclusions, which are based on much more rigorous testing.

The preprint, titled *Measures of reproducibility in sampling and laboratory processing methods in high-throughput microbiome analysis* finds these high-level results:

1. **Sampling method isn't that important**. Gut stool is not homogeneous, so you'd expect some variation in abundances depending on where and how you wipe, but when they systematically tested one person 11 times, they found the differences from the same

day were small. Samples taken the same day were 0.95+ correlated; those taken from the same individual on different days were 0.60+ correlated – much higher than the correlation between different people.

2. **Storage conditions don't matter (much) either** Whether you store the samples frozen, at room temperature, or in hot weather, your results won't be different enough to make them invalid.

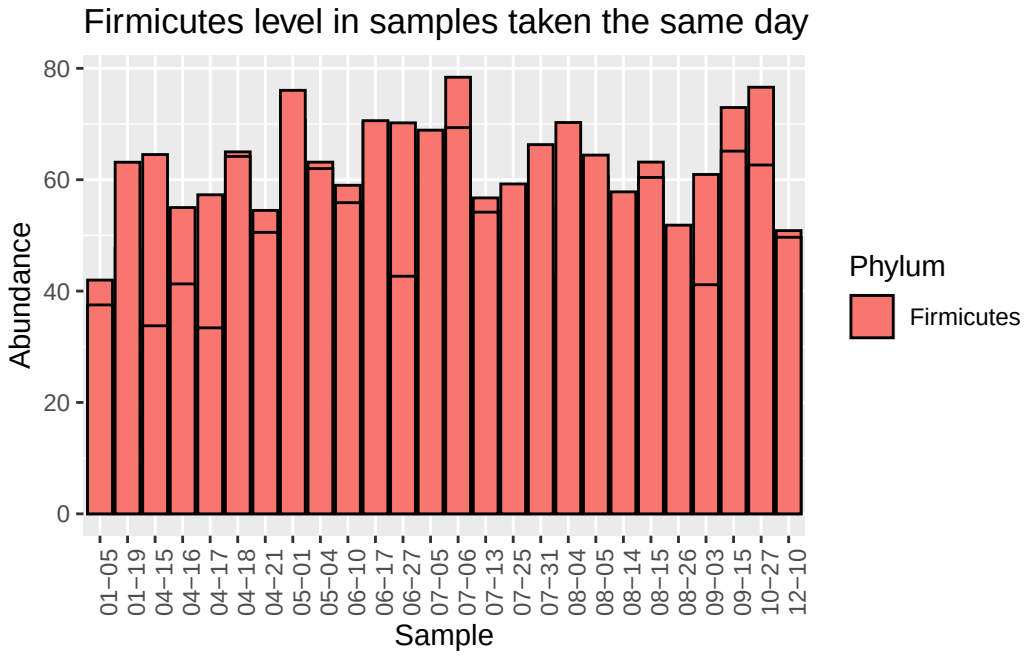
3. **Sequencing results are pretty consistent**. Turning a microbiome gut sample into usable data requires dozens of precise steps, any of which can potentially skew the results, but at least in uBiome's lab pipeline, the final results are highly reproducible.

All of this is good news to people hoping for important insights from their microbiome testing, but it still left me with some questions.

The paper doesn't describe exactly *how* they tested the person ("Subject A") who they found had consistent results over time. This is an experiment I've tried too – over 25 samples worth – and meanwhile several people have sent me the results where they happened to test twice. Can I replicate the uBiome results?

Let's start by looking at a single Phylum, *Firmicutes*, which is usually the most common in western guts. This is the highest-level taxanomical ranking as well, so the 16S method used in the uBiome pipeline should be pretty accurate. Using the 25 samples of which I have duplicates taken the same day, I'll compare the first sampling ("Sample1") with the second "(Sample2)".

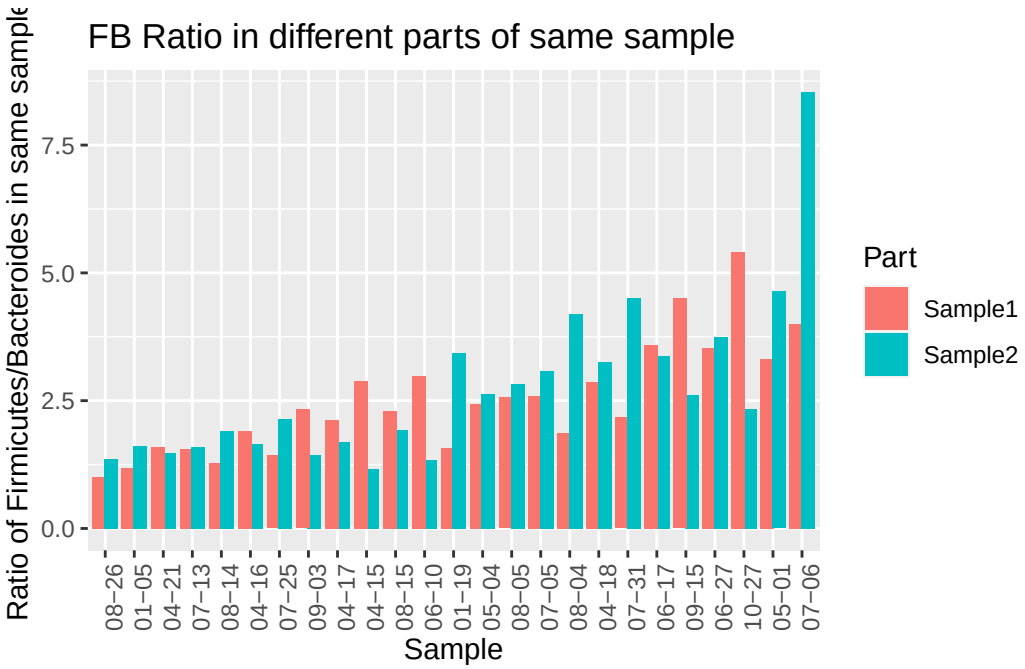
When we eyeball it, the *Firmicutes* doesn't appear to vary a whole lot between the same-day samples. The black lines in each of the bars is the level of *Firmicutes* found in the *second* sample. Although there are a few significant exceptions (10-27, 04-15, 6-10), most of the time the levels for this microbe seem pretty close no matter where you sample.



One limitation of the uBiome paper is that they only looked at a tiny subset of all the genus-level taxa found in the sample. Presumably they did this because they've previously shown that those particular genera are accurately represented in the sample, but if you want to know if something is evenly distributed, you can't rely on a subset. For example, even though a subset of my gut phylum, *Firmicutes*, is reasonably stable all on its own, the *ratio* of Firmicutes to other important taxa is all over the place.

Table 12.3: Lin's Concordance Correlation Coefficience for 25 samples

FB Ratio	Firmicutes	Bacteroidetes
0.4	0.39	0.5



Let's run the same correlation calculation that uBiome used:

At the phylum level, I find much less correlation (at best 0.50) than uBiome did (0.95). What are some possible reasons?

First, as noted they are looking at a subset of 28 taxa that they've decided can be most accurately detected using their pipeline. I'm looking only at one phylum. But *Firmicutes* is the most important, most broadly watched phylum in the gut. If this is measured inaccurately, what does that say about the rest of the experiments?

Second, although I'm studying only a single taxa, they're using a summary metric of *all* 28 taxa they measure. The paper doesn't explain how they summarize 28 microbial abundances into a single number, but I assume they are doing some common similarity metric, like Bray-Curtis. This is a simple and often-used way to tell how similar or different two vectors are from one another. I didn't do that because I'm comparing a single number, not a vector.

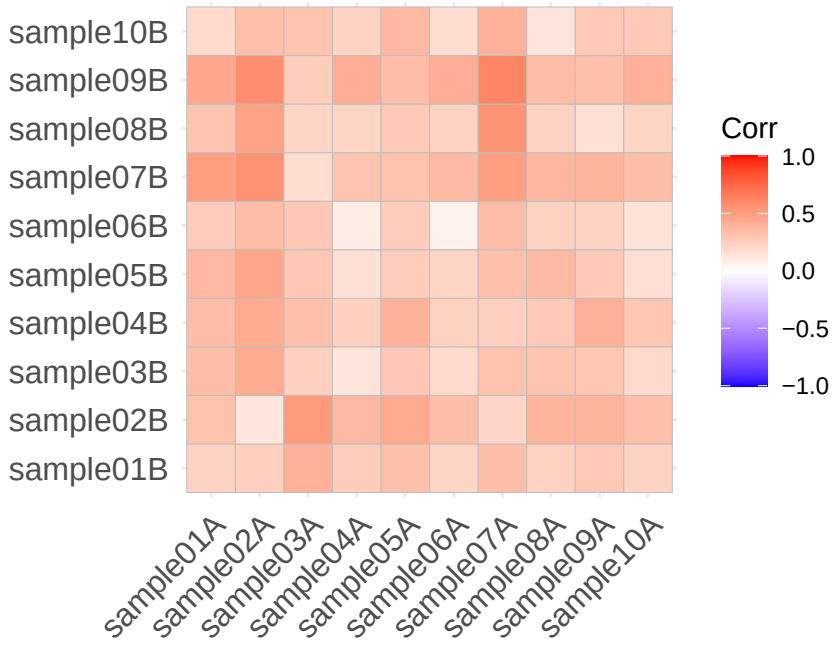
Let's see if I can make a rough estimate that would be similar to their list of taxa. uBiome's clinical test tracks 28 microbes at the genus and species level, not all of which can be seen in Explorer.

There are 12 genus-level taxa on both lists (Prevotella, Roseburia, Bifidobacterium, Alistipes, Odoribacter, Barnesiella, Campylobacter, Fusobacterium, Veillonella, Lactobacillus, Peptoclostridium, Salmonella, Ruminococcus)

Here are the correlations *between* the clinical taxa

Taxa1	Taxa2	Correlation
Fusobacterium	Prevotella	0.94
Peptoclostridium	Fusobacterium	0.67
Peptoclostridium	Prevotella	0.65
Peptoclostridium	Lactobacillus	0.64
Barnesiella	Alistipes	0.61
Veillonella	Fusobacterium	0.55
Odoribacter	Alistipes	0.54
Veillonella	Prevotella	0.52
Barnesiella	Odoribacter	0.50
Lactobacillus	Prevotella	0.44

Here are the correlations among the Bray-Curtis distances:



(which, upon reflection, means absolutely nothing)

Finally, I think the real difference has to do with sampling technique. I poke the swab all over the place into my samples. My guess is that their experimental subject probably swabbed the outside of the sample at two spots. That may or may not be more realistic than my method – it depends on whether you think toilet paper grabs only the outside or not – but

it *does* highlight the importance of consistency in how you take a sample. If, as the uBiome experiments appear to show, you sample only on the outside, then there is probably a lot of similarity in the same sample. If normal people are more like me, sampling all over the place, then my results show the variability may be much higher than uBiome thinks.

12.12 Conclusions

Are these results are meaningful enough to be worthy of further analysis? How representative were these samples of my microbiome at the time? I submitted only a tiny sample to the lab; are the bacteria distributed evenly enough that the size or location of the sample doesn't matter? Would I get similar results if I submitted two tests from slightly different sites on the same sample?

The answers to all these questions are unclear, but while we need to take these concerns seriously, my experience over many samples is that the results are consistent enough that, yes, the conclusions are actionable as long as we keep the limitations in mind. Here's why I think so:

First, my results are consistent with other "healthy omnivore" submissions that uBiome has received from others with diets and health histories similar to mine. I would be concerned if, for example, my firmicutes/bacteroidetes ratio were reversed.

Second, 16S technology has proven accuracy when identifying unique organisms, so I can generally trust information about the overall level of diversity regardless of specific proportions. Since diversity tends to correlate with health – and is manipulable based on what I eat – my own experience shows that the changes I see in these results go up and down consistently in the expected way. Even if specific points on my microbiome map are fuzzy, the overall landmarks appear to be solid.

13 My Tests

Microbe numbers shift daily in response to your environment, so a single sample won't give much more than a brief snapshot at a single point in time. But in over 600 tests under a variety of conditions, what did I find?

13.1 My Oral Microbiome*

The first place that microbes enter is also one of the richest and most variable environments in the body.

Scientists added an odorless compound from wine to a culture of known oral bacteria, and sure enough: the bacteria generated compounds that we can smell: terpenes, benzenic compounds and lipid derivatives. Each of us has a unique oral microbiome, and scientists were able to that that this inter-person variability is large enough to explain at least some of the differences in how each of us perceive a glass of wine.

What are the most important species in my mouth?

To microbes, your body looks like a hollow tube: skin on the outside, gut on the inside, and a mouth to allow passage between the two. Like purgatory, the mouth is a gatekeeper where new microbes wait before being whisked into the heavenly warm breeding grounds of the digestive system. But it's no easy waiting room either-the mouth contains many highly-distinct eco-systems, each as different from one another as the Sahara desert is to the bottom of the ocean. Most microbiome and genetic tests ask you to swab the inside of the cheek-an easy, straightforward place teeming with bacteria, but the bacteria in the cheeks can be very different from those on the tongue or the lips. I tested them all one morning right after waking up.

	Lips	Tongue	Cheek (Right)	Cheek (Left)
Streptococcus	57.36	6.44	38.41	43.42
Haemophilus	19.61	4.97	6.36	7.59
Gemella	8.47	2.23	10.67	12.64
Actinobacillus	3.07	0.26	3.91	3.32
Veillonella	2.14	7.70	1.81	2.20
Granulicatella	1.60	0.88	2.13	2.36
Neisseria	1.47	14.91	7.51	4.12
Fusobacterium	1.24	14.99	5.94	6.96
Porphyromonas	1.10	3.37	3.56	2.78
Rothia	0.90	0.21	6.49	2.83
Actinomyces	0.69	2.26	2.90	2.11
Prevotella	0.45	13.31	1.09	1.73
Alloprevotella	0.42	2.76	0.46	0.36
Leptotrichia	0.22	8.20	1.64	1.96
Capnocytophaga	0.20	3.87	0.59	0.64
Pasteurella	0.10	0.02	2.28	0.87
Lachnoanaerobaculum	0.04	1.19	0.09	0.19
Campylobacter	0.03	2.05	0.39	0.50
Johnsonella	0.02	1.43	0.07	0.12
Bacteroides	0.00	0.00	0.95	0.23

Dramatic differences in the types of microbes in each part of the mouth.

While there is some variation in the cheeks, there is a dramatic difference between them and the lips or tongue. Also interesting is the way the lips are dominated by just three taxa that make up more than 85% of the total abundance. In Inverse Simpson terms, the lips are the least diverse, whereas the tongue is the most diverse.

Regular testing of my microbiome often yields unexpected surprises, and this one has me stumped. Beginning in December 2016 and for no apparent reason, my mouth was colonized suddenly by a particular species of *Streptococcus* that had not been there before. Why? I'm not aware of any major lifestyle or other changes to cause this: same toothpaste, same living conditions. A few dietary experiments here and there, but nothing that coincides with these changes.

At the species level, I eliminated all samples with under 10,000 reads. We see something interesting: for no apparent reason, the species of *Streptococcus* detected in my mouth has changed. Suddenly, in December 2016 my mouth was colonized by a particular species that had not been there before. Why? I hadn't done anything special; I'm not aware of any major lifestyle or other changes to cause this.

I confirmed with the lab that it's not contamination. What's especially odd is that I experienced a shift like this twice now in one year. After comfortably floating along with Species

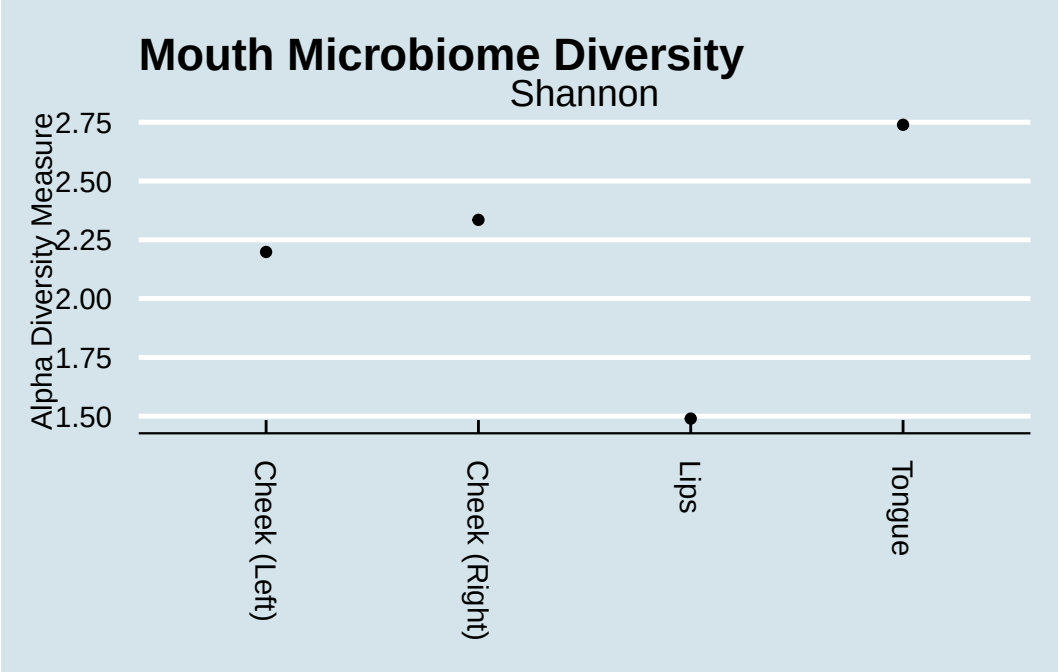


Figure 13.1: Even within the same mouth, a surprising variance in diversity.

Streptococcus sp. BS35a for more than six months, suddenly in August the balance shifted again, this time to *Streptococcus sp. 11aTha1*. Will it shift again? Who knows?

I confirmed with the lab that it's not contamination.

What's *especially* odd is that I experienced a shift like this *twice* now in one year. After comfortably floating along with Species *Streptococcus sp. BS35a* for more than six months, suddenly in August the balance shifted again, this time to *Streptococcus sp. 11aTha1*. Will it shift again? Who knows?

Earth's atmosphere was originally void of oxygen, a poisonous gas to the first, so-called "anaerobic" bacteria who thrived precisely because there was no oxygen. Over eons, as oxygen levels increased these microbes found places to hide: deep, dark pockets inside multicellular creatures who traded an oxygen-free interior for the abundant, exotic metabolites the microbes could synthesize. In humans, these bacterial safe-houses begin in the mouth, where the oxygen is low enough to keep the lights on for the anaerobes, while allowing occasional blooms for the aerobic bacteria that thrive whenever the mouth is open and they find fresh air.

Most of them do apparently need moisture: your salivary glands, strategically located in your cheeks and at the bottom of the mouth, churn out 1–2 liters of saliva per day.

The complexity of the mouth microbiome is compounded by the variety of surfaces, hard and soft, each with its own propensity to allow the formation of biofilms, tenacious clusters that

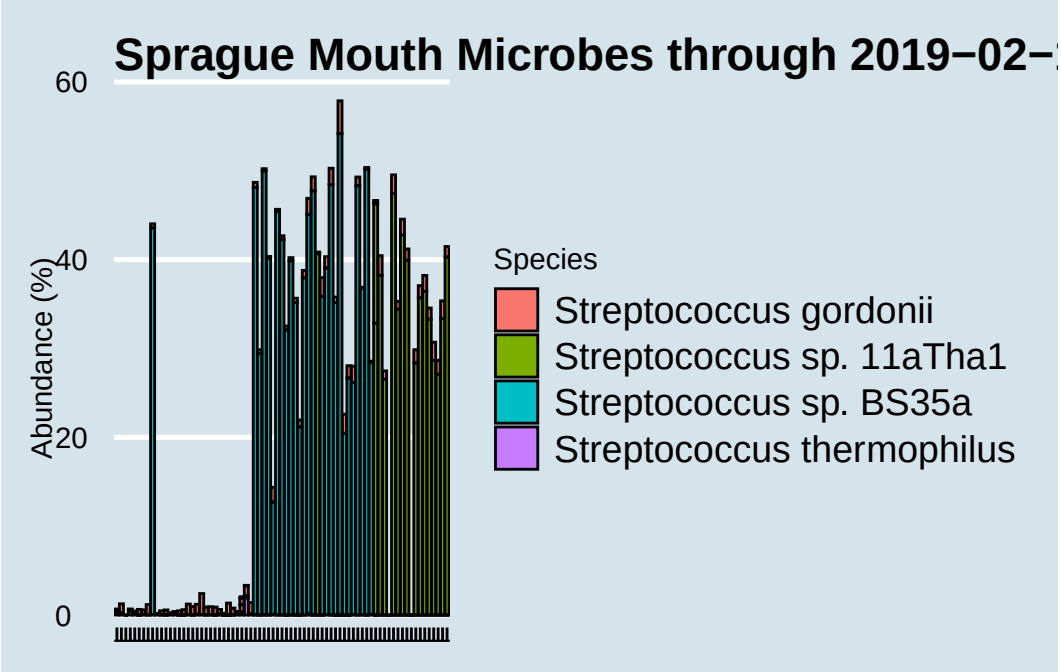


Figure 13.2: Odd shifts in some streptococcus species over time.

protect microbes against invaders. On teeth, we call it dental plaque, a favorite protective breeding ground of *Streptococcus mutans*, the cavity-causing villain that, once established, is hard to dislodge. I'm fortunate that my mouth microbiome appears to have none of this and it's true that I never have cavities. I've seen levels as high as 2% in some people, who have to visit the dentist no matter how much they brush.

Most microbes go down the hatch to the stomach, of course, but overly-aggressive tooth brushing or dental work can let a few can sneak into the bloodstream directly, where they can find their way to the lungs, the liver, or the heart, sometimes with deadly consequences. The "viridans" streptococci are one well-studied example: beneficial in the mouth, they outcompete other streptococcus enough to prevent strep throat, yet are the leading cause of heart valve infections if they make it into the bloodstream.

These mouth microbes have other interesting properties that may affect much more than we think. When scientists added an odorless compound from wine to a culture of known oral bacteria, the bacteria generated compounds that we can smell: terpenes, benzenic compounds and lipid derivatives.[1] Each of us has a unique oral microbiome, and the experimenters were able to show that this inter-person variability is large enough to explain at least some of the differences in how each of us perceive a glass of wine.

So what about these odd new ones that showed up in my mouth?

[1] Muñoz-González et al. (2015)

I've looked up their names in every reference I can think of, but have found nothing. That's not too surprising: about a third of oral microbes are known only by their gene sequences.[2] The most satisfying answer from a microbiology expert I consulted is that these are likely to be "passenger microbes", doing nothing in particular helpful or harmful.

In other words, like so many other microbes in our environment, they are just along for the ride.

13.2 My Nose Microbiome

Springtime for many people brings hay fever, an allergic reaction known to be associated with the microbiome. I fortunately don't suffer from the condition, but I wondered if maybe I could find something in my nose microbiome that would show a seasonal shift, perhaps something aligned with allergy season. Even if I don't have symptoms, maybe by finding some of the key microbes involved my data might be useful to others who would like to explore more of the link between their allergies and microbes.

Like every place on the body, your nose has its own unique microbial ecology, as different from other sites as a tropical rainforest is from the artic tundra. Unlike the gut or the mouth, your nose is in constant contact with the external environment, exposed to new microbes that float in day and night with every breath you take.

It seems reasonable to expect that we'd see different microbes floating in our environment as the seasons shift. After all, changes in temperature, humidity, and daylight affect the abundance and variety of plants, so of course these shifts will affect microbes. But is there a pattern to the changes?

To find out, I sampled my nose microbiome more than 50 times over a period of three years, carefully tracking the date and microbial species in each sample. In all, I found more than 200 different (genus-level) bacteria from about 350 unique species.

Using a versatile clustering algorithm called non-metric multidimension scaling (NMDS), I calculated the statistical correlations among the hundreds of microbes in a way that let me build a two-dimension chart where similar samples are clustered together, and less-similar samples are further apart. Where there are significant differences among samples, an NMDS chart will show obvious clustering, with similar samples bunched together and separate from other clusters.

I couldn't see any patterns when I generated an NMDS clustering diagram on the whole data set, which includes samples taken in multiple geographies. When I looked only at those samples taken in a single geography, my home , the results were a little more, well, consistent with a theory that seasons matter. (Figure 13.3)

[2] https://www.nature.com/articles/sj.bdj.2016.865.pdf

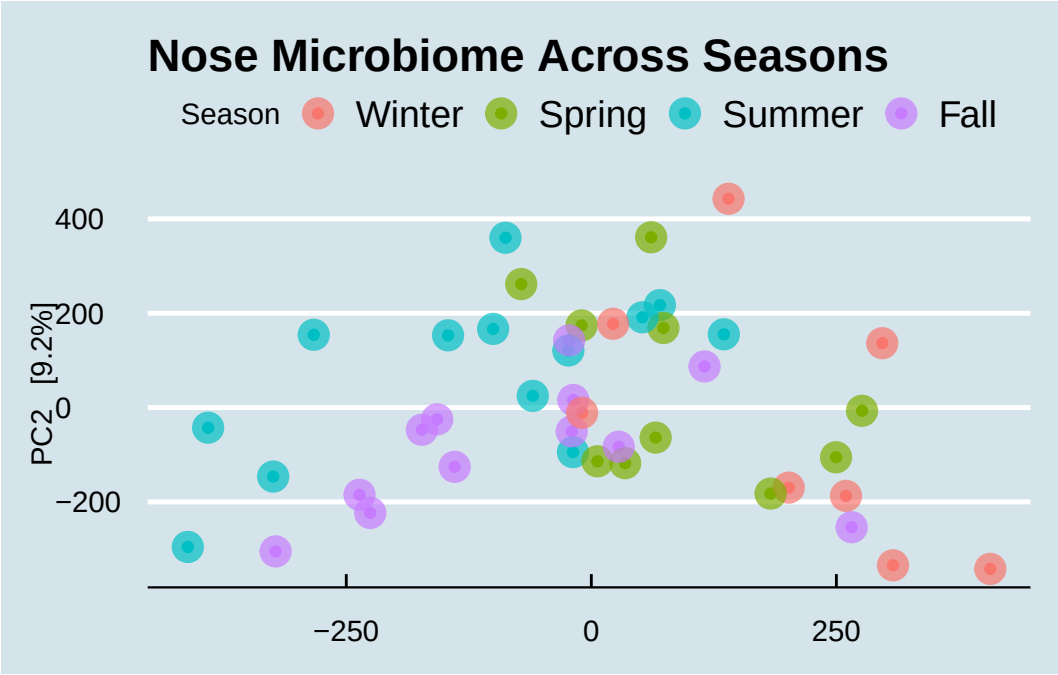


Figure 13.3: Nose microbiome across seasons in a single geography.

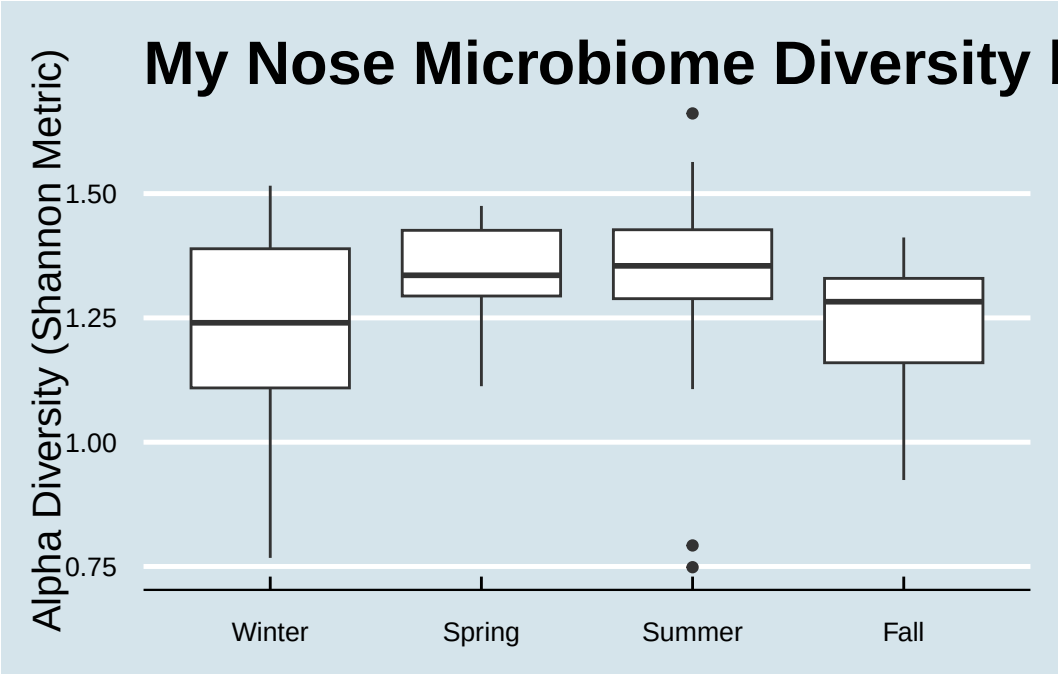


Figure 13.4: Nose microbiome diversity in a single geography by season.

Okay, this chart is kind of a mess. If there were major shifts, we'd see clear clusters. Although we don't quite see that, it *is* interesting that the seasons *do* kinda-sorta hang together with much overlap. For example, the (red) winter dots are all on the right side of the chart, with the (blue) summer dots mostly on the left. The (purple) fall dots are, if anything, closer to the winter samples, and (green) spring is closer to summer. Both spring and fall were more in the middle, which you might kinda-sorta expect given that they are generally less extreme than the other two seasons.

Importantly, each of the samples was taken under the same conditions: I swabbed the nostrils for about 30 seconds right after waking up each morning. Since my bedroom is kept at a climate-controlled temperature year-round, these samples wouldn't show direct results of major seasonal changes, though microbes in my bedroom are presumably affected somewhat by what's happening outdoors. I also have a number of samples taken in different geographies while traveling, but I removed them before making this chart.

What about the richness and diversity of microbes throughout the year? The box plot in Figure 13.4 shows that summer has the widest diversity *range* (those dots outside the main box are outliers). In this plot, the area in the box represents 50% of the samples, and the line through the middle is the median diversity.

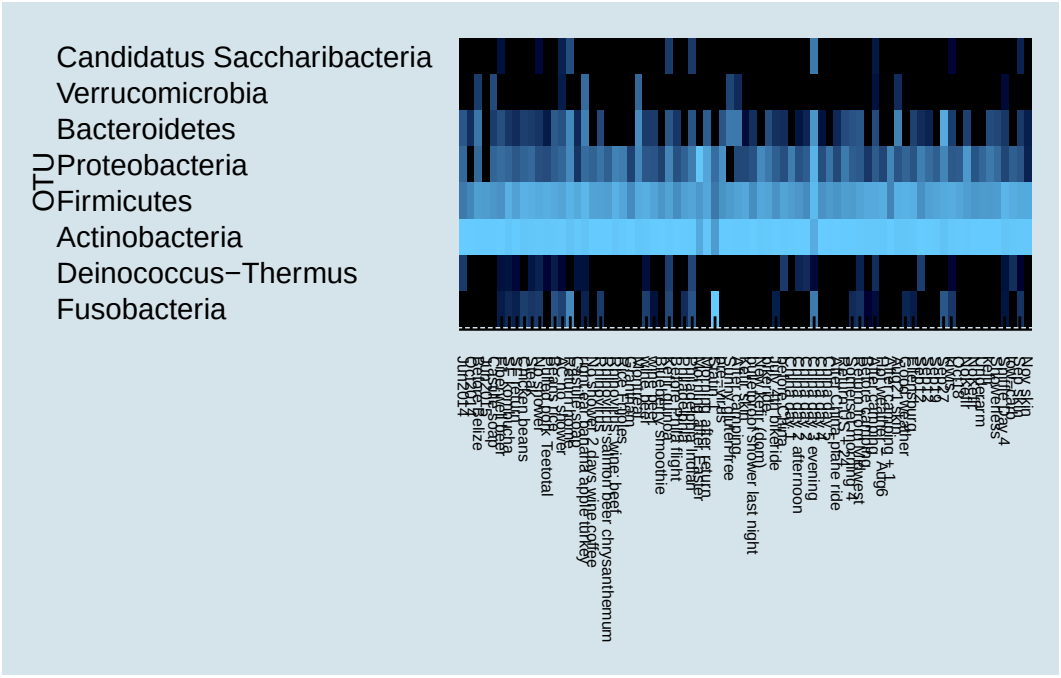
I spend more time indoors in the winter, so perhaps that explains why median diversity is lowest then. Again, it's interesting that both spring and fall have similar diversities to each other, perhaps because both seasons have similar amounts of indoor-outdoor time and maybe the variety of microbes reflect that.

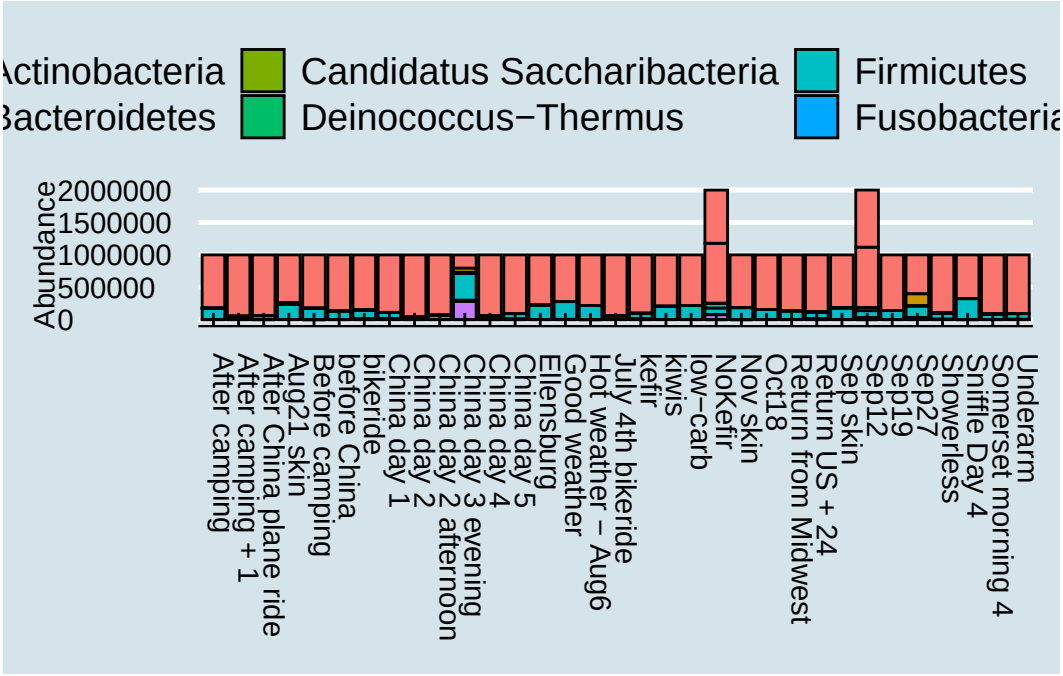
Is it just me? Can I learn anything by comparing my nose microbes to other people? I ran several analyses against other people but so far haven't found much.

13.3 My Skin Microbiome*

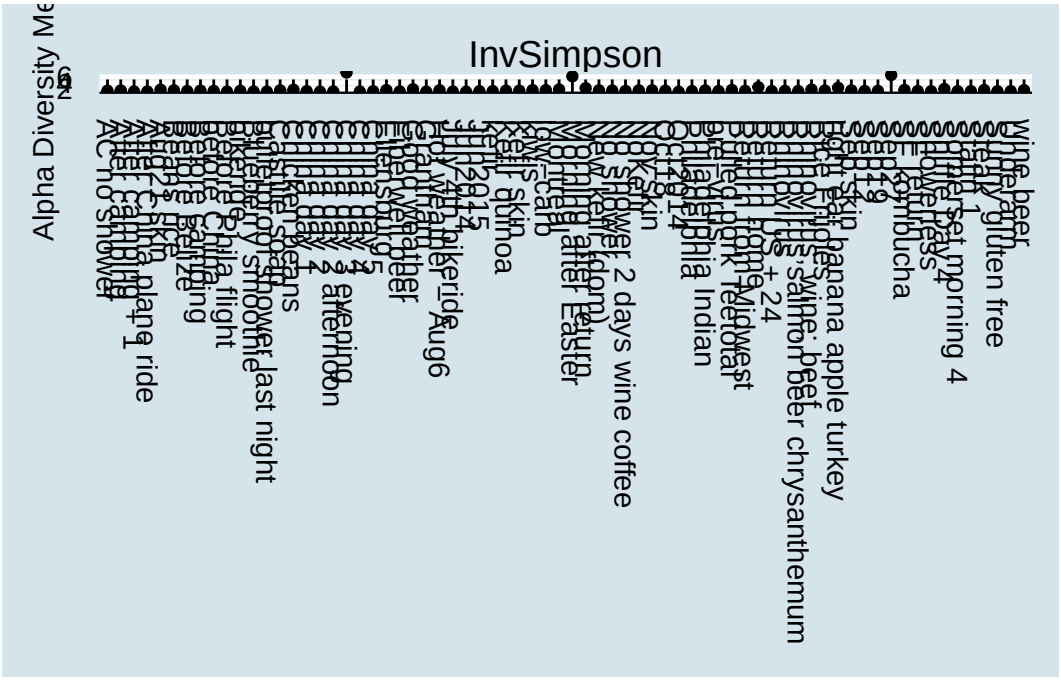
Microbes of the gut are important, but many other organisms are crawling all over you too. What can we learn from studying my skin samples?

Like the gut, the vast majority come from only two genera.



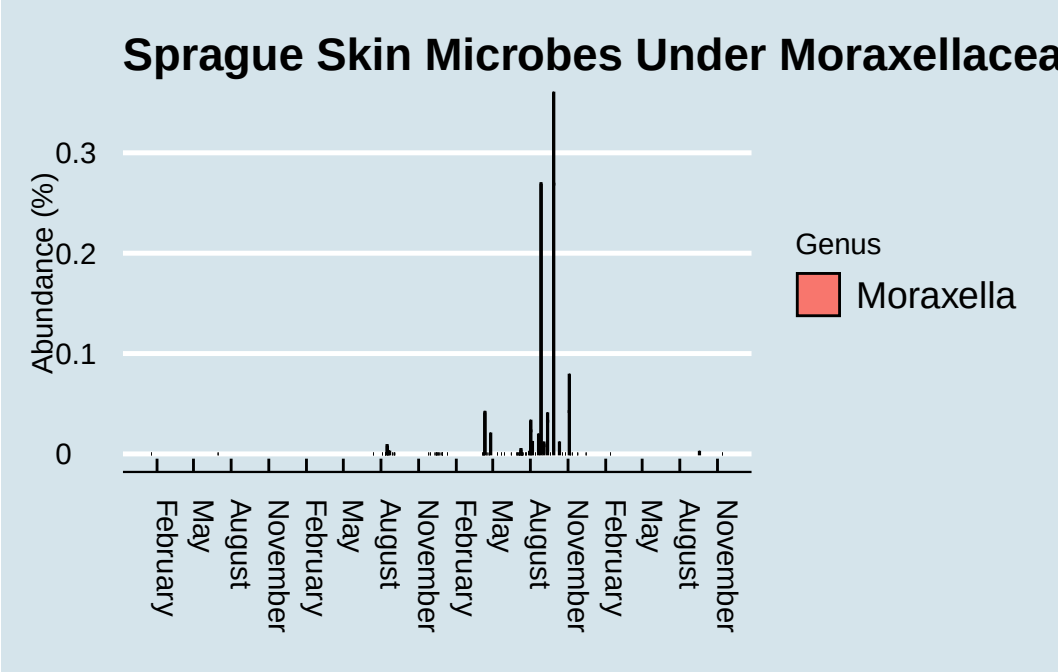


And here's the diversity:



Is it just me? I also looked at the many other samples people have sent. Like me, most of them are dominated by *Actinobacteria* and *Firmicutes*, with a tiny bit of *Proteobacteria* and even smaller amounts of others. At the genus level, the vast majority are *Propionibacterium* and *Staphylococcus*, with an occasional spike of that smelly *Corynebacterium*.

A recent paper published in Nature warned that several pathogens are lurking in common kitchen sponges. In particular, it noted the abundance of *Moraxellaceae*, a common skin bacterium. How much do I have?



Apparently I have a small amount, depending on when I sample.

Now look at an odd new bacteria that showed up in some of my samples from Spring 2017 (Figure 13.5)

Not sure what this could be doing.

13.3.1 Body odor

That unpleasant smell from you underarms is caused by *Corynebacteria*[3]. Perhaps there is a relationship between what I find behind my ear and other parts of the body. Let's check (Figure 13.6)

I also tested the difference between behind-the-ear (the normal way) and on the forehead (Figure 13.7)

And the underarm versus behind the ear (Figure 13.8)

Once again, sampling site matters: there is a significant difference in the type of microbe behind the ear compared to the forehead. Incidentally, notice in the underarm the much higher abundance of *Corynebacterium*, which produces that distinctive smell of body odor.

See the Appendix for an overall summary of my skin experiments

[3]See more at Rob Dunn Lab or the original academic paper here: Natsch et al. (2003)

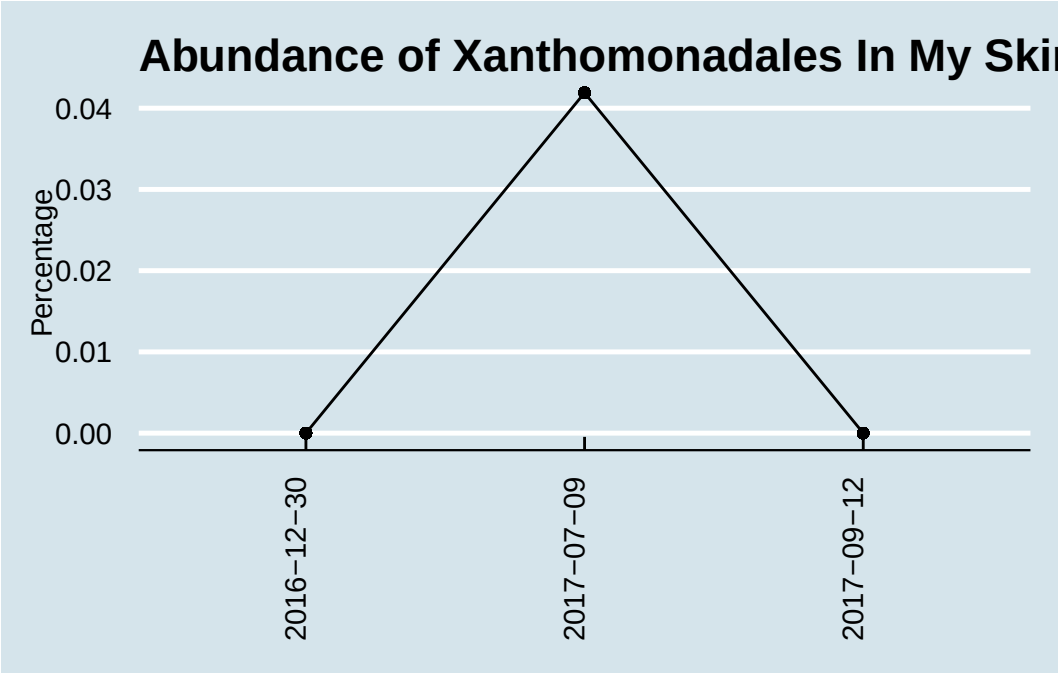


Figure 13.5: Levels of Xanthomonodales spiked for some unknown reason.

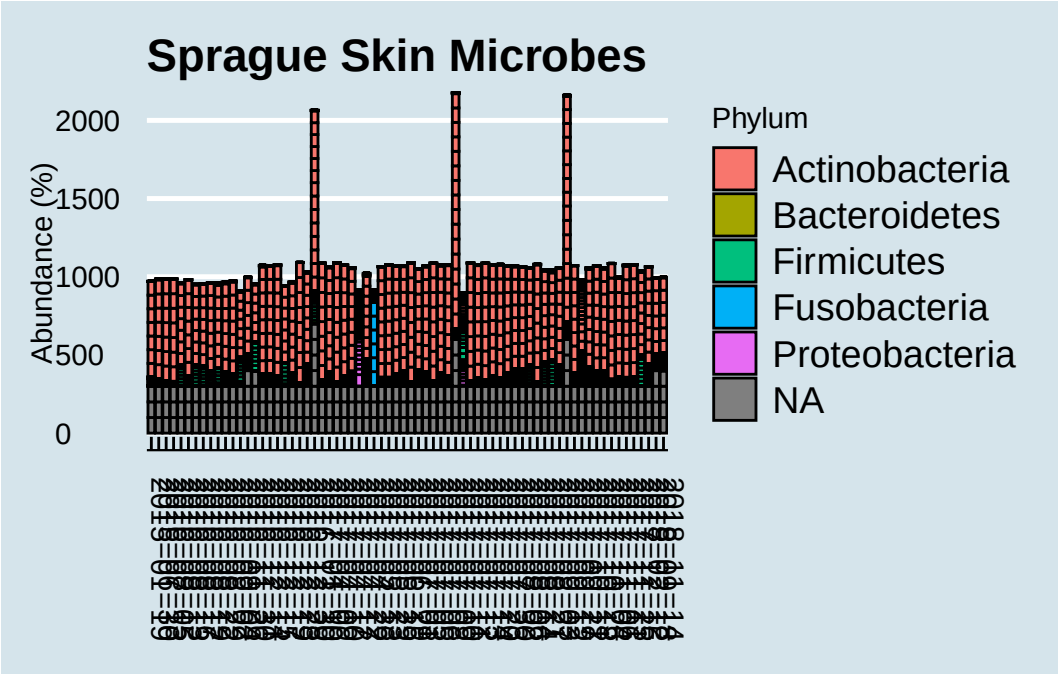


Figure 13.6: Skin microbes, phylum level, overtime

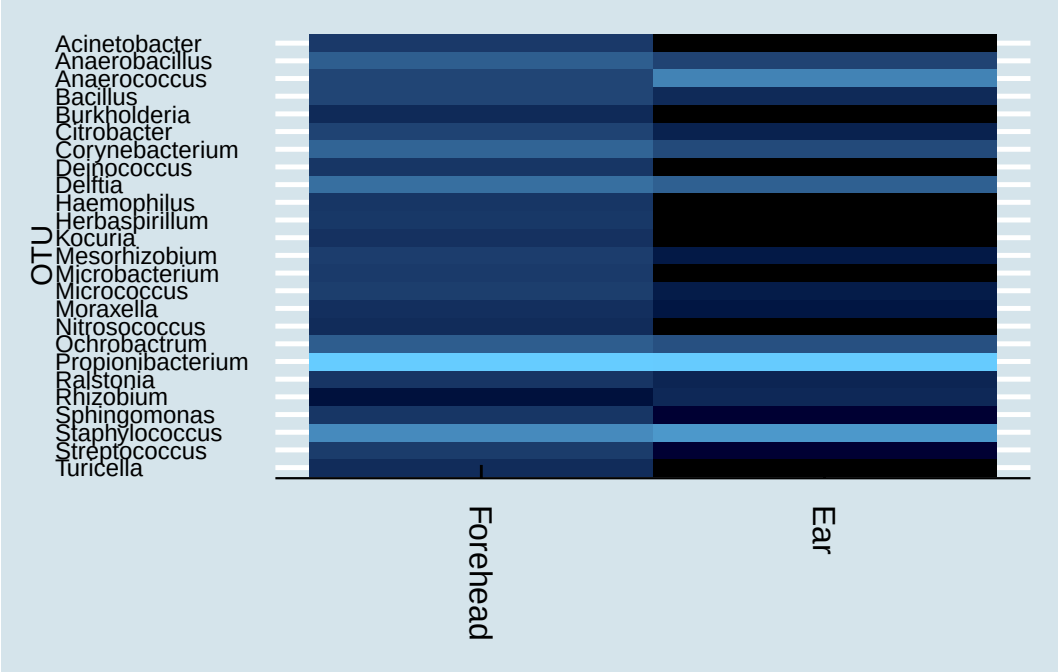


Figure 13.7: Forehead and ear nave compared. (Lighter shades are more abundant)

Table 13.1: Comparing Skin Samples

	Forehead	Ear
Actinobacteria	92.9342	88.1038
Firmicutes	4.9351	11.1056
Proteobacteria	2.0409	0.7800
Deinococcus-Thermus	0.0489	0.0000
Cyanobacteria	0.0217	0.0000
Bacteroidetes	0.0190	0.0104

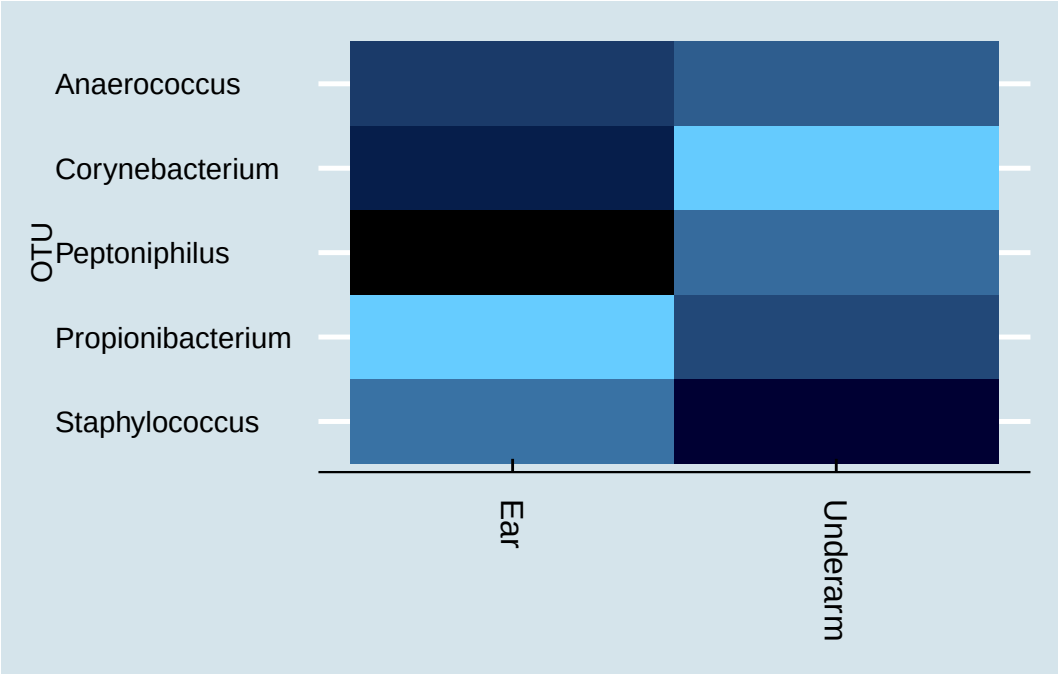
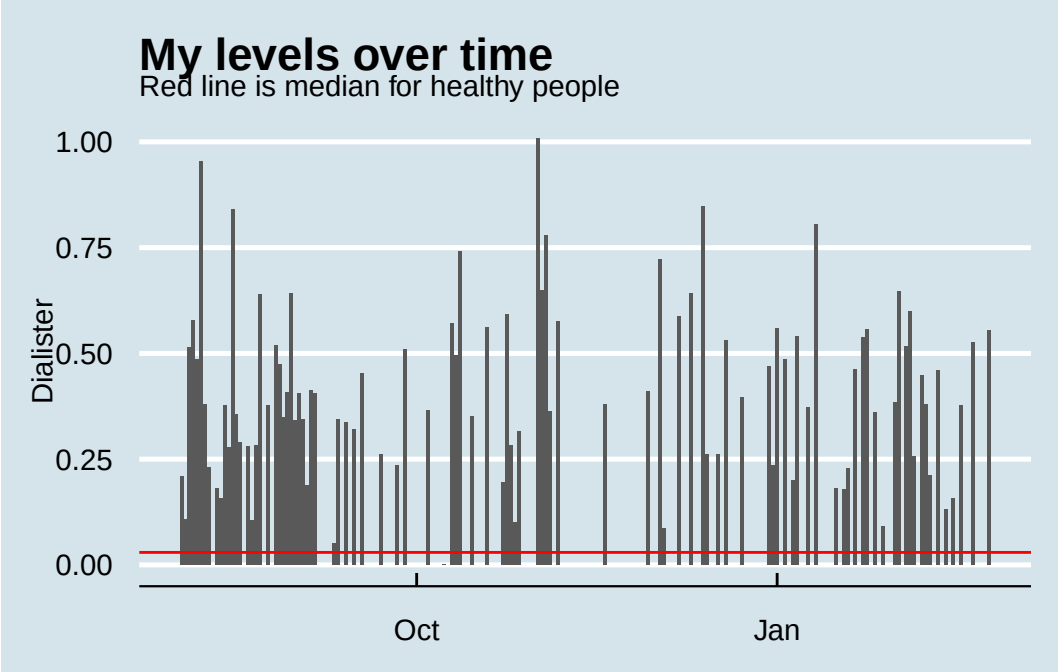


Figure 13.8: Skin samples from two sites: the normal behind-the-ear and from the underarm. Lighter colors are *higher* abundance

13.4 Are my Dialister levels normal?

A well-done 2019 study found that people suffering from depression have significantly lower levels of two groups of bacteria, *Dialister* and *Coprococcus*, possibly due to a potential ability of the gut microbiome to synthesize 3,4-dihydroxyphenylacetic acid, a breakdown product of the neurotransmitter dopamine[4]

How are my levels of *Dialister*?



13.5 Omega-3 and the microbiome**

This paper suggests a relationship between *Lachnospiraceae* family and Omega-3. I tried taking high-omega 3 fish oil pills for a week. Figure 13.9

Another study from the University of Nottingham found that omega-3 correlates with the microbiome in women.

[4]https://www.nature.com/articles/d41586-019-00483-5

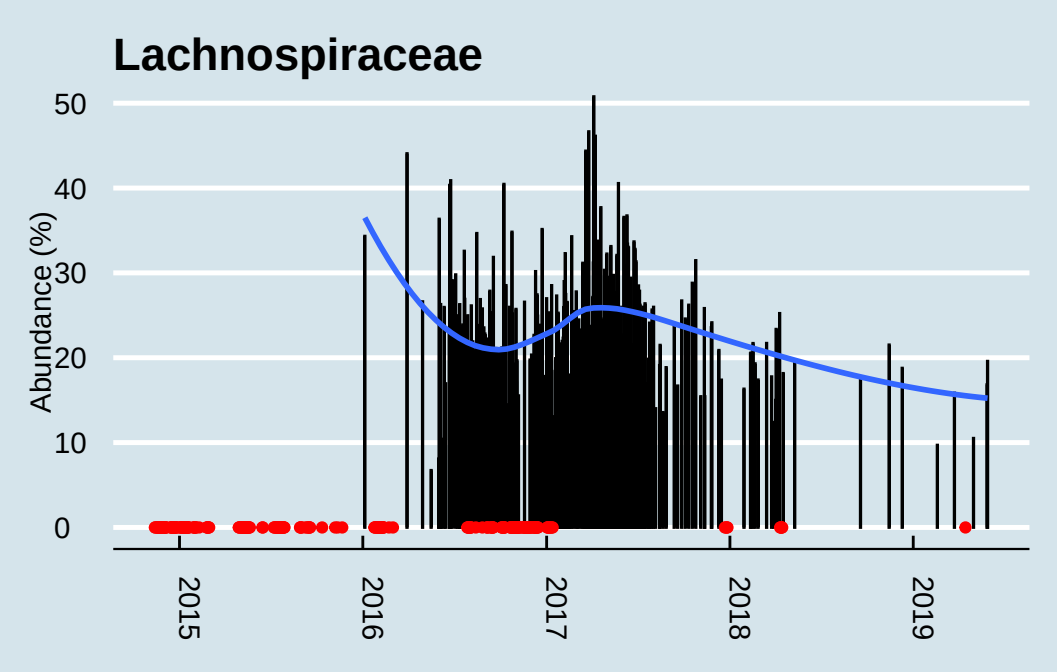


Figure 13.9: Days marked in red are days I took two fishoil capsules.

13.6 Soylent

A team of undergraduates at the University of California Berkeley conducted an experiment with 14 people to see if the nutrition drink Soylent would change the microbiome[5]. They found that it increased the ratio of Bacteroidetes to Firmicutes by a significant amount. How about me?

Interestingly, looking back through my daily microbiome samples to see which dates I tried Soylent, I got this: (Figure 13.10)

The red dots are dates when I drank Soylent.[6] Unfortunately, the samples failed on two of the dates in this chart, so I'm unable to see how my gut microbiome looked immediately before taking the Soylent, but still, isn't it strange that my F/B ratio was reasonably stable until then?

The shift is more dramatic if we look at a longer time frame, the weeks before and after the Soylent drinking (Figure 13.11)

[5]Hsu et al. (2017) and see the $6,405 crowd-sourced campaign that funded it

[6]More precisely, the sample taken that day represents the food I ate the day *before*. In other words, the red dots are the *day after* I drank Soylent.

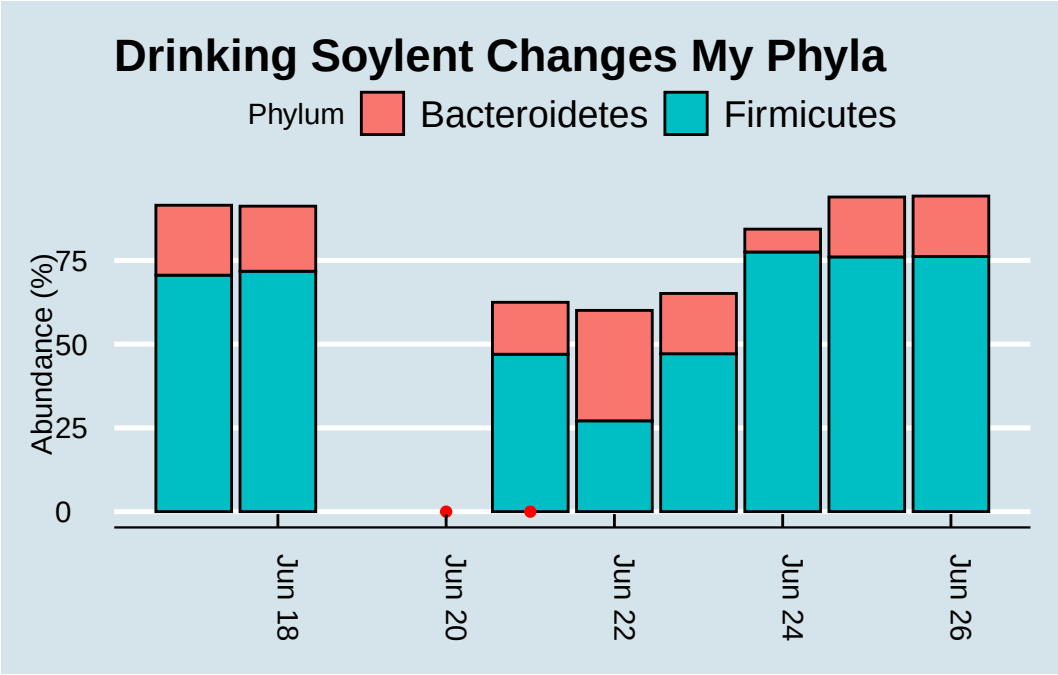


Figure 13.10: Red dots mark days when I d#| rank Soylent.

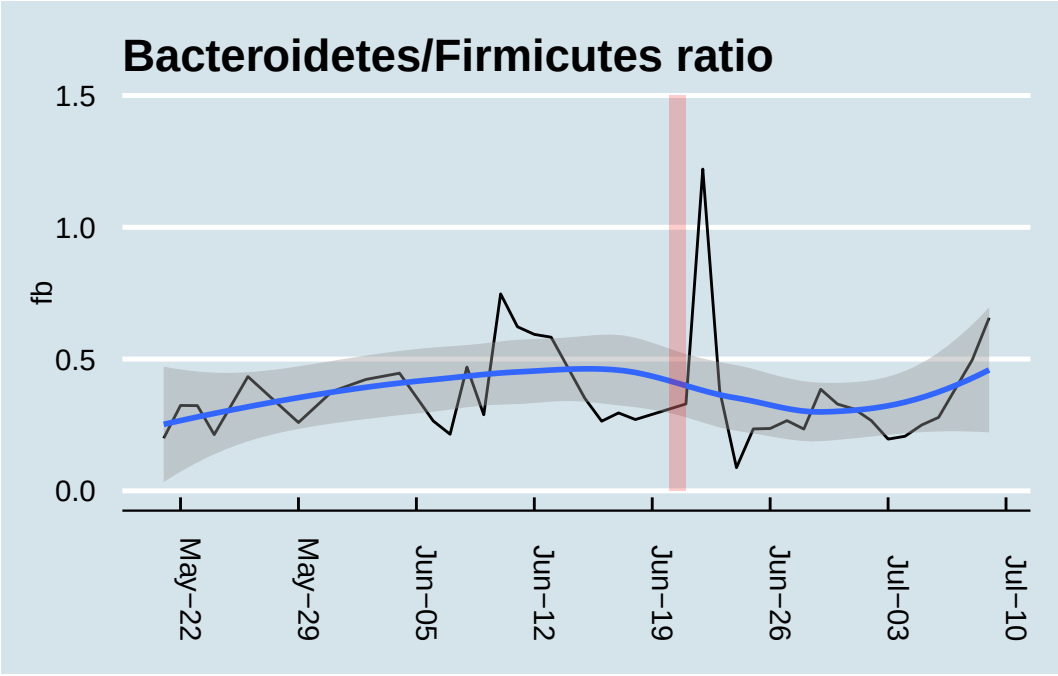


Figure 13.11: Soylent-drinking d#| ays are highlighted in red.

This is by no means a confirmation of the results of their experiment, since mine was just an *ad hoc* test for two days among many other types of food-eating and tests that I regularly conduct on myself. That said, it is odd that I find a significant shift in that ratio, in the same direction as in their published trial.[7]

Why would this be?

The nutritional label gives some possible clues. (Figure 13.12)

One of the main ingredients, maltodextrin, is a man-made polysaccharide popular as a food additive for its usefulness as a thickener and texturizer. Usually synthesized from corn or wheat, it has been added to food products since the 1950s and is now in something like 60% of all packaged foods. It also has some well-known effects on the microbiome[8], at least in mice, and on the ability of some bacteria to form biofilms. I couldn't find any studies in humans that specifically look at the affect on the microbiome, except now this Berkeley study.

The Soylent web site explains that the maltodextrin is there to provide carbohydrates. Mixed with oat flour and other fibers to give it an overall lower glycemic index, it's naturally easy to digest and a quick source of energy. That sounds like a recipe that should significantly affect the microbiome, especially if you use it, as intended, as your main source of food.

Interestingly, my gut diversity seems to have increased sharply right after drinking the Soylent, followed by a crash a few days later.

The diversity calculated in this chart is a very crude measurement that tries to summarize a complex ecology into a single number, and as you can see it tends to vary sharply from day-to-day anyway. That said, it's not *that* variable over time, and the few days after Soylent seem notably higher than the rest of the period measured. I'm betting this is really caused by the fact that I was visiting another city at the time, so the increase is likely related to travel more than the food itself. Still, something for future research to consider.

Finally, let's look at the overall phyla-level breakdown. (Figure 13.14)

Interestingly, the days after Soylent drinking show that the Firmicutes has been replaced by *Verrucomicrobia*, the phylum that contains *Akkermansia*. The affect lasts a few days, and it's unusual compared to the rest of the sampling period, so I doubt it's a coincidence. Still, it's very hard to tell the cause.

More details are available on the Mycrobes site of the student group that did the experiment. There is also a lively Reddit discussion.

[7] Important technical note: the study authors didn't use the standard uBiome bioinformatics pipeline (like I did), choosing instead to build their data from the raw FASTQ files returned from the uBiome 16S sequencing lab. That would normally make a significant difference in the results, so compare my data points with caution. Still…

[8] See Nickerson, Chanin, and McDonald (2015)

soylent United States/Canada 2.0

Nutrition Facts

Serving Size 1 bottle Soylent (414 mL)
Servings Per Container 1

Amount Per Serving	
Calories	400
Calories from Fat	190
	% Daily Value*
Total Fat 21g	**35%**
Saturated Fat 2g	**10%**
Polyunsaturated Fat 2.5g	
Monounsaturated Fat 16g	
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 300mg	**13%**
Potassium 700mg	**20%**
Total Carbohydrate 37g	**12%**
Dietary Fiber 3g	**12%**
Soluble Fiber 1g	
Sugars 9g	
Protein 20g	

Vitamin A 20%	· Vitamin C	20%
Calcium 20%	· Iron	20%
Vitamin D 20%	· Vitamin E	20%
Vitamin K 20%	· Thiamin	20%
Riboflavin 20%	· Niacin	20%
Vitamin B6 20%	· Folic Acid	20%
Vitamin B12 20%	· Biotin	20%
Pantothenic Acid 20%	· Phosphorus	20%
Iodine 20%	· Magnesium	20%
Zinc 20%	· Selenium	20%
Copper 20%	· Manganese	20%
Chromium 20%	· Molybdenum	20%
Chloride 15%		

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Potassium		3,500 mg	3,500 mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 · Carbohydrate 4 · Protein 4

Ingredients: Water, Maltodextrin, Soy Protein Isolate, High Oleic Algal Oil, Isomaltulose, Canola Oil, Rice Starch, Oat Fiber, Isomaltooligosaccharide, Soy Lecithin, Potassium Chloride, Calcium Phosphate, Magnesium Phosphate, Natural & Artificial Flavors, Dipotassium Phosphate, Salt, Choline Chloride, Gellan Gum, Sodium Ascorbate, dl-alpha-Tocopheryl Acetate, Ferrous Gluconate, Vitamin A Palmitate, Zinc Sulfate, Niacinamide, Sucralose, Calcium Pantothenate, Thiamine Hydrochloride, Copper Gluconate, Manganese Sulfate, Riboflavin, Pyridoxine Hydrochloride, Vitamin D, Potassium Iodide, Chromium Chloride, Biotin, Folic Acid, Sodium Molybdate, Phytonadione, Sodium Selenite, Vitamin B12. **Contains: Soy**

Manufactured for Rosa Labs
207 S Broadway Suite 600
Los Angeles, CA 90012

While not intended to replace every meal, Soylent can replace any meal.

Children, women who are pregnant, nursing, or may become pregnant should consult their doctor before consuming Soylent. Please refer to soylent.com/notes for more information.

Soylent™ is a trademark of Rosa Labs

Figure 13.12: Nutritional label and ingredients list from a bottle of Soylent.

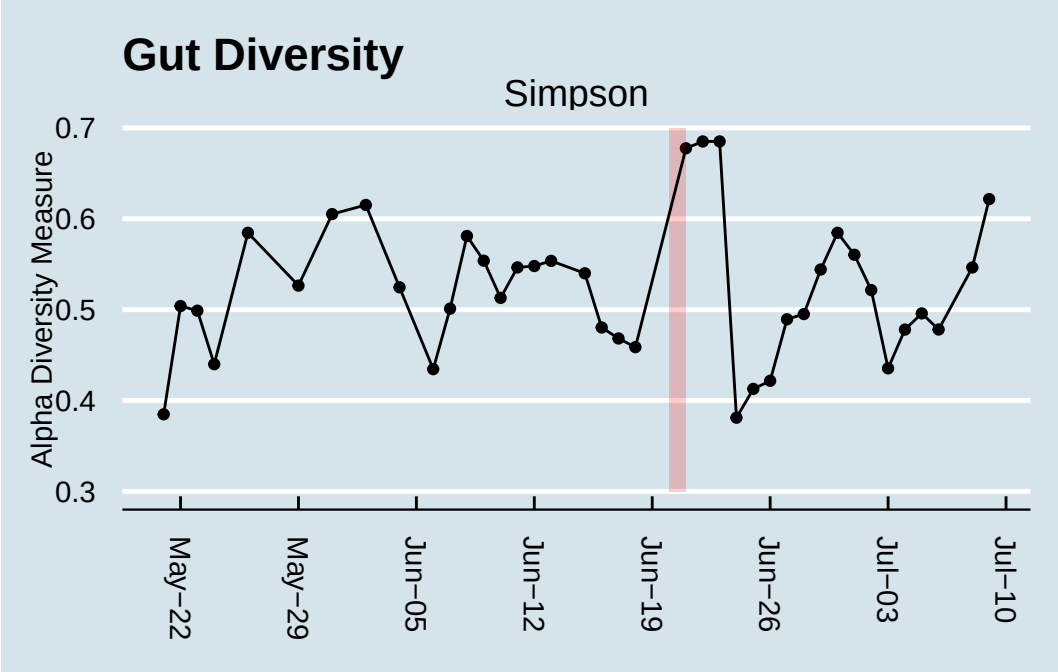


Figure 13.13: Family-level diversity. The period highlighted in red is days I drank Soylent.

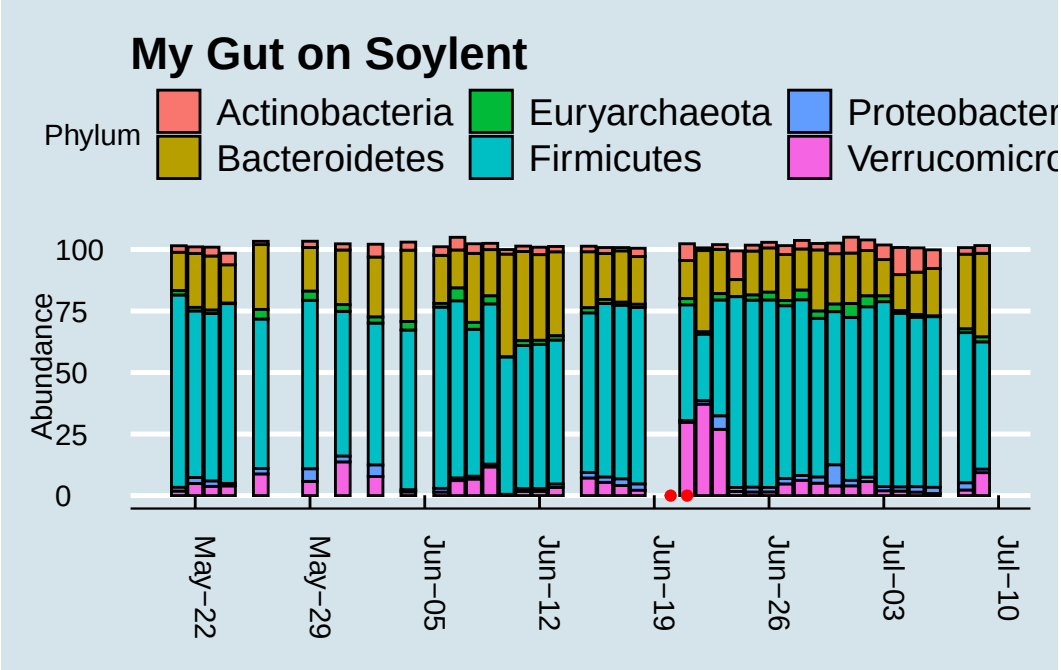


Figure 13.14: Phyla breakdown shows high Verrucomicrobia after Soylent drinking. Genus is Akkermansia.

14 Case Studies

Hundreds of people have sent me their microbiome results. Here are some examples showing how I walk through specific cases.

14.1 Healthy People

> Healthy people are all alike; every unhealthy person is unhealthy in their own way. - Leo Tolstoy *Anna Karenina*.

A statistically-generated NMDS diagram is an easy way to show hundreds of samples at one time. Samples that are more similar to one another are shown closer together, forming "clusters" that can give us an idea of which people are most similar. (Figure 14.1)

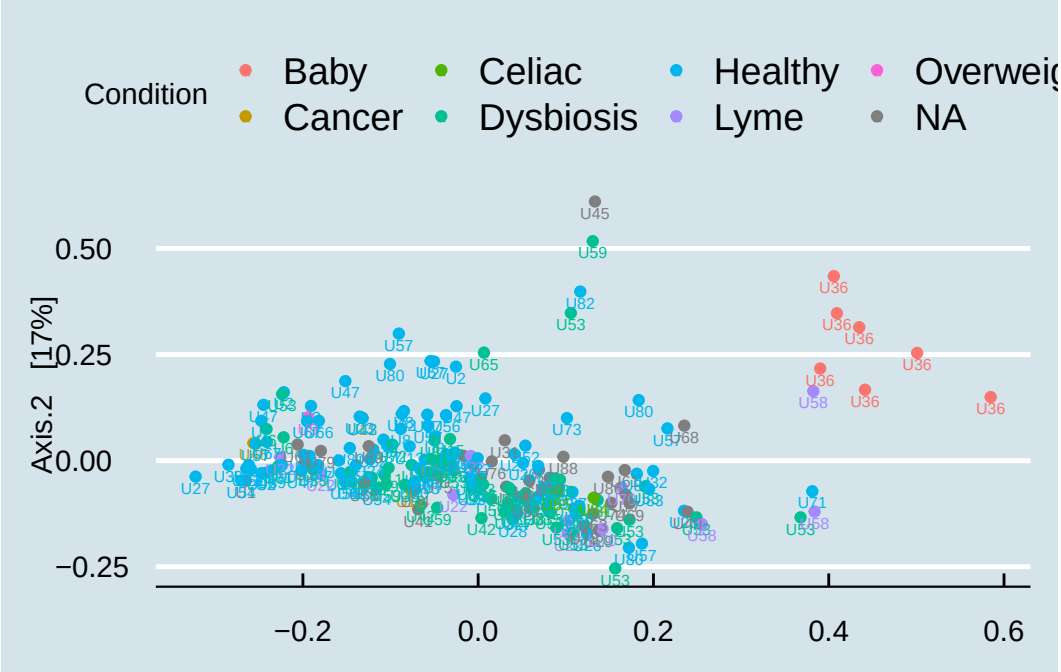


Figure 14.1: Overall clustering of hundreds of unique samples in my database.

The differences here are not strikingly obvious, but you can see a cluster among the baby samples. There is overlap, but it appears that the healthy people are generally in a separate space from the people who self-report some type of dysbiosis.

What if we look just at the "healthy" people? In Figure Figure 14.2 I unfortunately don't see any special clusters.

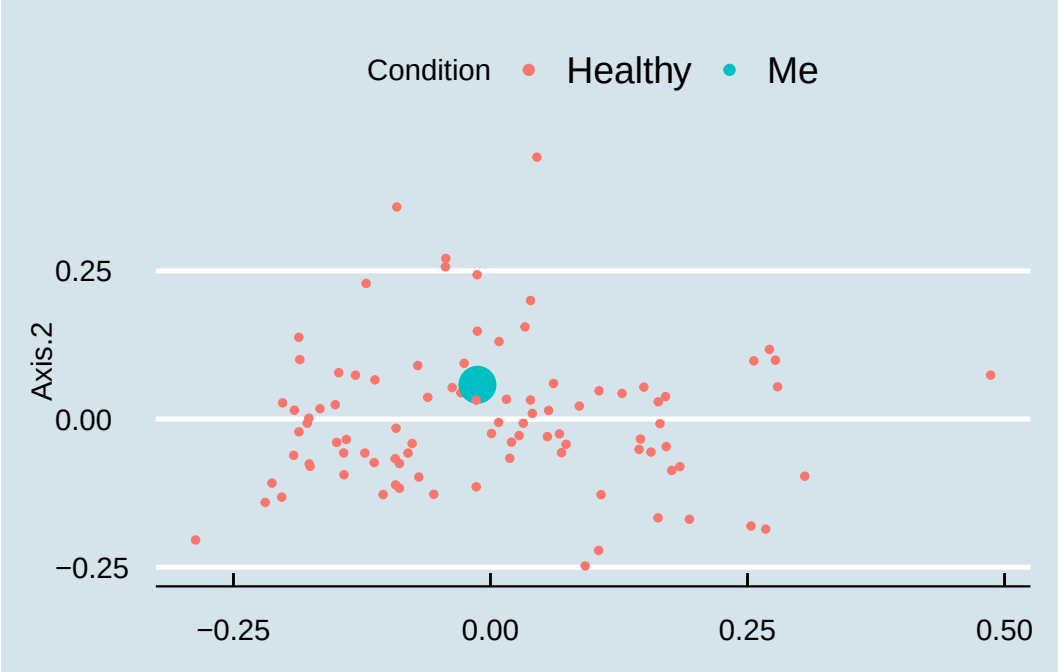


Figure 14.2: An overall look at clustering among just healthy people.

14.2 Family members

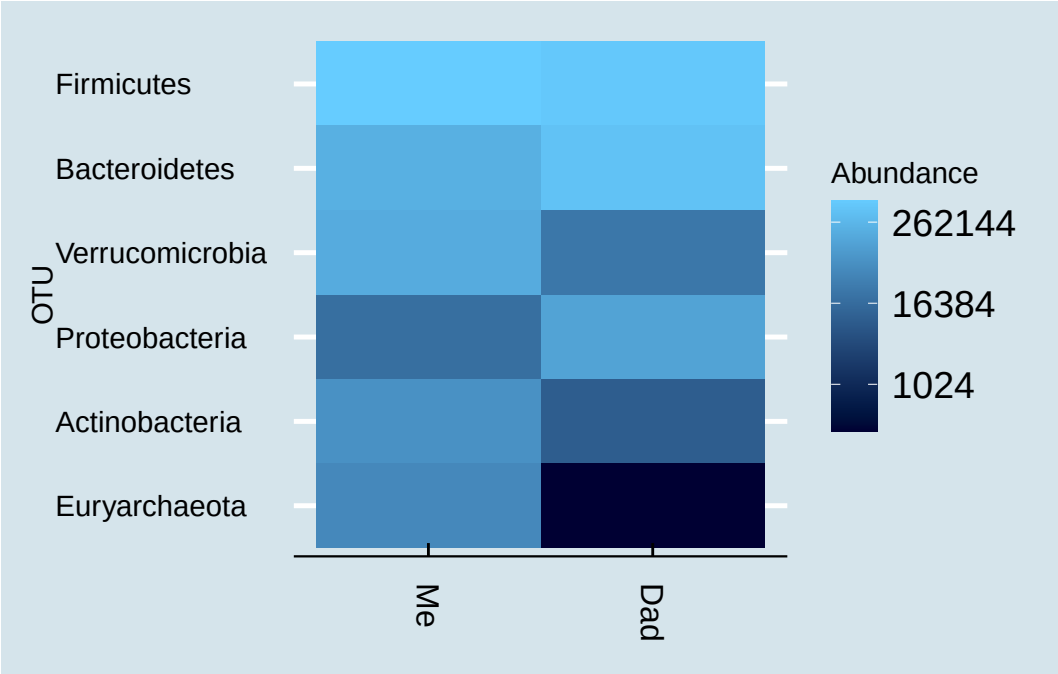
My father and I live in different parts of the country: he's in the Midwest (where I grew up) and I'm on the West Coast. We're both healthy omnivores and, other than a couple-decade age difference we both eat roughly similar foods and have similar medical histories.

That said, I was surprised to see our gut biomes were so similar. Here's how I did the comparison.

Remember that a gut microbiome varies *a lot* day-to-day, depending on whatever food we happened to eat, exposure to illness, geographic location, even time of year. To keep the variables as constant as possible, I'll compare two samples taken on the same day:

	Me %	Dad %
Firmicutes	53.82	46.44
Bacteroidetes	19.80	37.50
Verrucomicrobia	16.80	2.44
Actinobacteria	6.65	0.91
Euryarchaeota	4.78	0.02
Proteobacteria	1.79	12.43

At the highest, phylum, level, we can see the percentage abundances look different. One easy way to spot specific patterns is through a heatmap, like this one:



Here are some of the taxa that are unique to me:

	Me (%)
Pyramidobacter	0.42
Dialister	0.38
Cloacibacillus	0.32
Methanomassiliicoccus	0.09
Gordonibacter	0.04
Hydrogenoanaerobacterium	0.04
Mogibacterium	0.02
Megasphaera	0.01
Sporobacter	0.01
Acidaminococcus	0.01

Taxa found in my father but not in me.

	Me Species(%)
Bacteroides plebeius	2.38
Odoribacter laneus	1.04
Dialister propionicifaciens	0.37
Butyricimonas virosa	0.29
Anaerotruncus sp. NML 070203	0.21
Parabacteroides johnsonii	0.20
Bacteroides salyersiae	0.19
Collinsella sp. GD3	0.09
Roseburia hominis	0.06
Gordonibacter pamelaeae	0.04

Taxa found in my father but not in me.

The list includes the familiar *Bacteroides plebeius*, the "seaweed-digesting" microbe which we've discussed previously. Dad never lived in Asia, so this is not a surprise.

and some that Dad has that I don't:

That *Thalassospira* is a mystery. A quick literature search reveals nothing, but it's abundant enough to make me wonder if there's something special about Dad's lifestyle that would harbor it. Note that it's not visible on the species level, an indicator that nothing is known below the genus.

I tried to esimate, roughly, how common it is among the other samples I've seen: It ranges between zero and `10.1979`

The vast majority of samples have none, but it's not unknown either:

Since Dad has already submitted several samples, I can also check whether and how much he has in his other samples:

Table 14.1

	Dad (%)
Paraprevotella	1.30
Butyrivibrio	0.39
Robinsoniella	0.15
Citrobacter	0.07
Catenibacterium	0.05
Arthrobacter	0.02
Parvibacter	0.02
Brevibacterium	0.01
Holdemania	0.01
Campylobacter	0.01

	Dad Species (%)
Desulfovibrio piger	2.50
Akkermansia muciniphila	2.17
Sutterella stercoricanis	1.50
Alistipes putredinis	0.92
Sutterella wadsworthensis	0.65
Phascolarctobacterium sp. 377	0.34
Paraprevotella clara	0.21
Bacteroides clarus	0.18
Collinsella aerofaciens	0.07
Parabacteroides goldsteinii	0.03

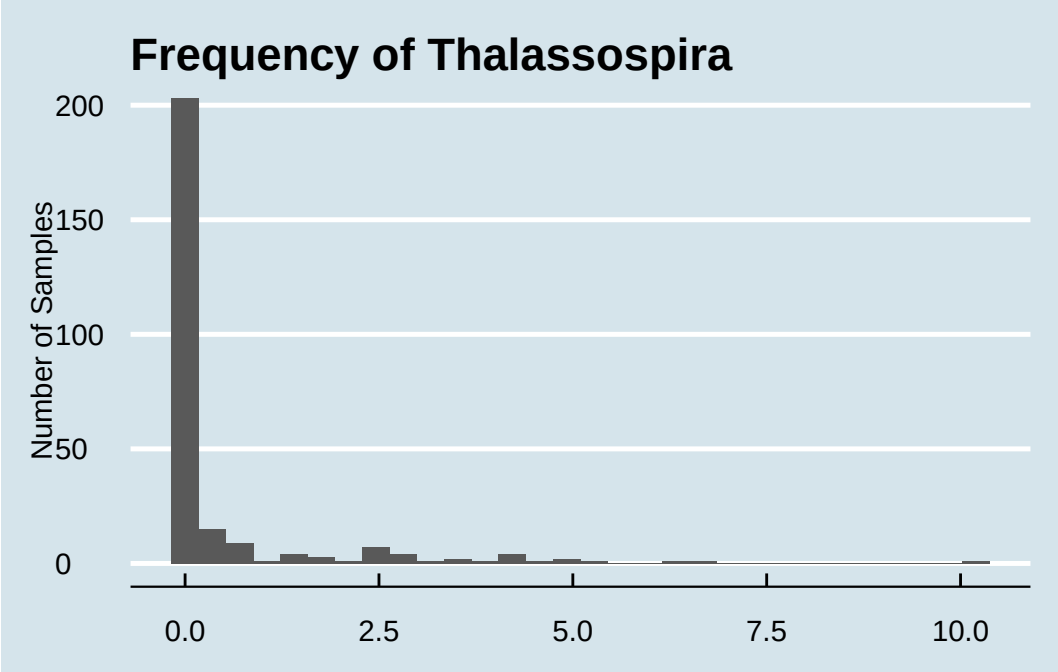
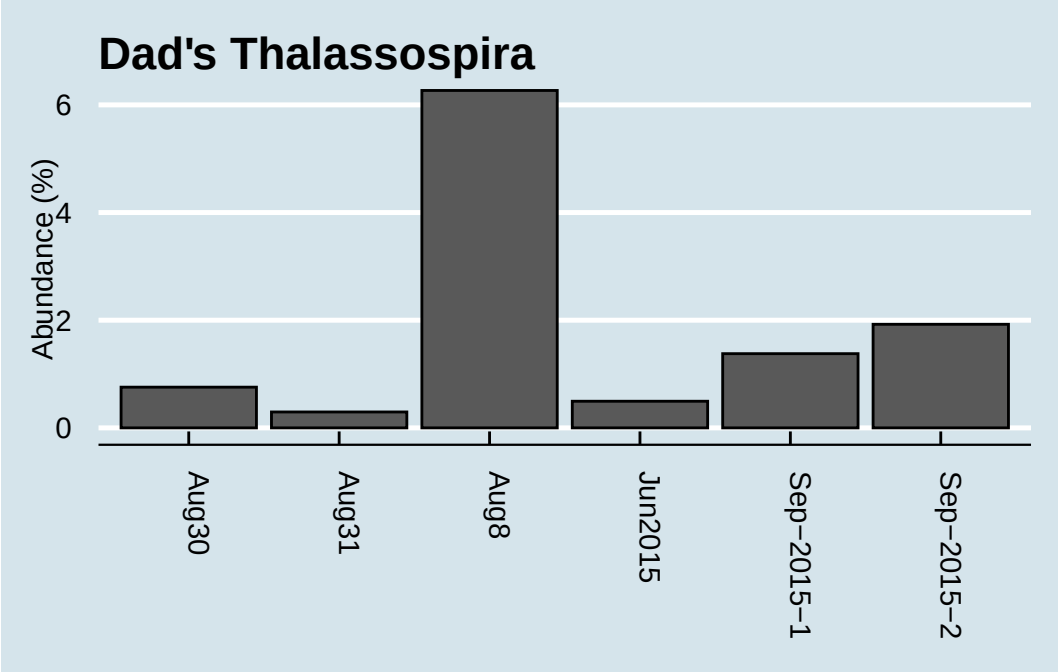


Figure 14.3: How common is Thalassospira?



The answer is that he has *some* in all of his gut samples. This particular one was unusually

high, but without more data it's hard to tell if that's significant.

14.2.1 My sister

Now let's compare myself with my sister. She made two samples (A and B), but the first thing we note is how different they are from each other. Despite being taken a month apart, they look significantly different.

	A	B
Firmicutes	39.74	60.61
Bacteroidetes	30.89	34.30
Actinobacteria	17.12	2.41
Verrucomicrobia	9.82	0.17
Synergistetes	1.24	0.40
Proteobacteria	0.90	2.08

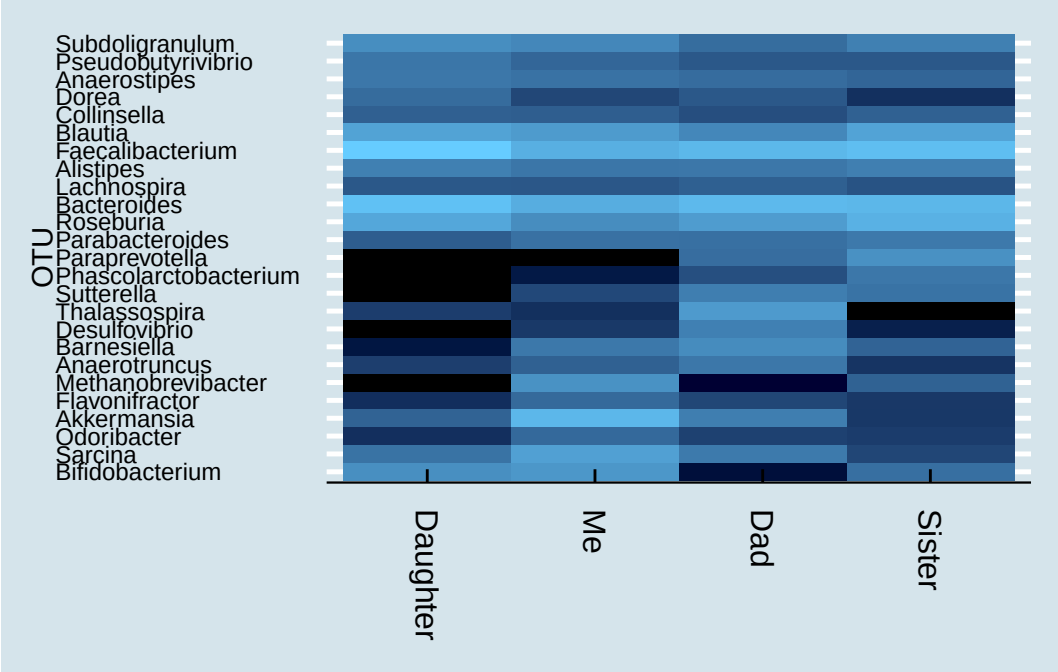
Sample A is the oddball, with unusually low Firmicutes than Sample B, and different from both me and our father, despite eating roughly the same diet. Can we try to undersand what's driving the difference? Let's look at the genus level:

	A	B
Bacteroides	10.27	16.29
Porphyromonas	9.91	0.00
Akkermansia	9.82	0.17
Anaerococcus	8.81	0.00
Varibaculum	7.73	0.01
Corynebacterium	6.11	0.00
Peptoniphilus	5.96	0.00
Blautia	1.85	8.43
Faecalibacterium	0.67	21.13
Roseburia	0.19	13.53

Aha! the *Porphyromonas* is the giveaway. That taxa almost never appears in a gut sample. In fact, of the hundreds of samples I've studied, it was nearly zero in all but the gut samples. I think we can safely assume that my sister's sample A was contaminated somehow.

Finally, let's make one big comparison among all my family members

	Me	Dad	Sister	Daughter
Akkermansia	16.74	2.35	0.17	0.88
Faecalibacterium	12.81	16.84	21.13	32.95
Bacteroides	11.82	17.89	16.29	22.70
Sarcina	7.72	2.03	0.30	1.57
Blautia	6.39	3.26	8.43	8.40
Bifidobacterium	5.60	0.03	1.37	4.21
Roseburia	3.93	6.73	13.53	9.80
Subdoligranulum	3.26	1.26	2.51	4.12
Barnesiella	1.91	3.82	0.90	0.04
Alistipes	1.75	1.85	2.42	2.54



Presented this way, a few items stand out:

My daughter is very different from the rest of us, missing whole classes of microbes. Like me, she is missing *Paraprevotella*.

Dad's low *Bifidobacterium*, probably a function of age, may explain some of his sleep troubles. Fortunately he has *some* Bifido, so I'm optimistic that with the right diet he may be able to increase his levels. My sister, who complains of poor sleep too, has low Bifido as well, but again the good news is that it's not zero: she has something to work with.

14.3 Alzheimers and the microbiome

"Anne" is a 40-year-old mother with a secret: her 23andme genetic test results show she is homozygous for the APOE-4 variant, which in slightly-misleading-but-you-know-what-I-mean everyday language means she has the gene for Alzheimer's disease. Statistics show that about 80% of people like her will develop the condition, and with this particular gene variant, it's likely she may start to see early symptoms as soon as age 50. Scary! No wonder she doesn't want anyone to know, including her children and relatives.

But Anne is also an optimist: she prefers to see herself as one of the 20% with the gene who *won't* develop any symptoms. And she's ready and motivated to do whatever necessary — diet, exercise, lifestyle changes — to beat this thing. She also knows that her body includes much more than human DNA, that for every human gene like that APOE-4 variant, she is host to as many as ten or a hundred times as many microbial genes, including — perhaps — some that with a bit of nurturing might help offset or prevent whatever propensity her human DNA has to this terrible disease.

Scientists researching Alzheimer's disease have uncovered some intriguing relationships with the microbiome. (see *this recent New York Times article* for a summary.) Some early AD symptoms, like a loss of smell, may be clues that the brain has been attacked by something that came from outside. The microbiome of the mouth, especially, is an excellent hiding place for low-grade infectious agents thanks to its many dark corners with regular access to both

the inside and outside of the body[1] . After reading about these relationships, Anne submitted several oral samples and shared the results with me.

The AD research field has blossomed lately with the realization that the brain, once thought to be completely sterile, is home to many microbes. This discovery and additional research has excited the editors of the respected *Journal of Alzheimers Disease,* who concluded a recent issue[2]:

> We propose that infectious agents, including HSV1, *Chlamydia pneumonia*, and *spirochetes*, reach the CNS (Central Nervous System) and remain there in latent form. These agents can undergo reactivation in the brain during aging, as the immune system declines…The consequent neuronal damage… occurs recurrently, leading to (or acting as a cofactor for) progressive synaptic dysfunction, neuronal loss, and ultimately Alzheimers Disease.

That's a powerful indictment of specific microbes, and the article calls them out by name. So does Anne have any in her sample?

Unfortunately, here's where we see both the promise and the limitations of those of us who suspect the microbiome will play an important role in eventually conquering this terrible disease.

The promise is intriguing: if we could identify the specific microbes underlying the condition, and then, perhaps through antibiotics or probiotics or some other intervention, what if we could get rid of the "bad" microbes and reseed with the "good" ones?

Here's a high-level (phylum) look at Anne's oral microbiome:

	1	2	3
Firmicutes	69.76	42.14	59.15
Bacteroidetes	14.58	13.52	5.80
Verrucomicrobia	8.30	0.00	0.00
Euryarchaeota	7.52	0.00	0.00
Actinobacteria	5.41	3.70	0.37
Proteobacteria	1.94	36.47	28.63
Synergistetes	0.12	0.00	0.00
Fusobacteria	0.01	4.20	1.08
Candidatus Saccharibacteria	0.00	0.08	5.07
Spirochaetes	0.00	0.01	0.02

Careful readers will immediately notice the Spirochaetes in those two later samples – the same microbe identified as a suspect in the *Journal of Alzheimer's Research.* Is this just a coincidence?! Or have we found a link?

[1] See more here: https://www.semanticscholar.org/paper/The-microbiome-and-disease%3A-reviewing-the-links-the-Shoemark-Allen/89b2295bab63c6d9267dc29198cb829f452efb51?tab=citations

[2] Itzhaki et al. (2016) and here:https://content.iospress.com/articles/journal-of-alzheimers-disease/jad160152

At this point, (big groan), we know there are quick-buck charlatans out there who will seize on an observation like this to sell hope to Alzheimer's sufferers and their families: how about a new anti-Spirochetes supplement? A seven-step "detox" plan to permanently rid your system of Spirochetes? Great idea for a new business, or maybe a best-selling book, right?

Unfortunately there are no shortcuts, and real conclusions from this data are still a ways away.

It turns out that Spirochetes is actually quite common in the oral microbiome. It's a broad category of free-moving bacteria that like to hide in low-oxygen environments. Its most infamous members include the genus *Troponema*, associated with syphilis, which come to think of it is a disease that affects the brain. (In botany class they like to joke that it's called Spirochaete because that's what you get when you cheat).

The Spirochaetes in Anne's test results are not *Troponema*, but even if they were it wouldn't mean much. A lot of people have these. I have some in my own mouth microbiome. The ecology of the mouth is so rich and complex that it's almost never possible to identify something as either "bad" or "good". Remember the example from earlier of "viridans" streptococci, the ones that beat back Strep Throat but can also cause heart problems?

The same is likely to be true about whatever microbes might be involved with Alzheimer's. But the good news is that more sampling can play a role in helping to narrow down the microbes that are different in people who go on to develop the disease. If we can collect enough samples from people like Anne, who have a family history and are at high risk for AD, we can compare them to one another as well as to thousands of samples of people who are normal risk and maybe we'll see a pattern.

For example, when Anne compared her mouth biome results with those from a close relative, she found that she has these unique phyla. The relative does not have them:

Anne's Unique Phyla	% diff
Candidatus Saccharibacteria	0.08%
Spirochaetes	0.01%
Tenericutes	0.00%

Interestingly, this relative has none of her Spirochaetes. And we find two others missing as well. Do they matter? Who knows?

The microbiome studies that have been conducted so far on AD patients are too limited to offer suggestions for what Anne can do right now, but slight differences like this offer her some ideas for possible experiments in the meantime.

Anne already follows the general advice that doctors give to everyone, including those at risk of AD, who wants a healthy microbiome: get plenty of exercise and sleep, eat healthy

unprocessed foods, and avoid antibiotics. But, just possibly, there are variations on these general good habits that might help her today.

For example, she's experimenting with different toothpastes to see how that affects her mouth microbiome. Did you know that most of the common toothpaste brands include powerful antibiotics?[3] Could the difference in brand be responsible for the unique phyla she sees? To learn more about herself, she's experimenting with alternate brands – testing her oral microbiome before and after to see the effects.

This is not the end of the story. Sadly we don't know what will ultimately happen to Anne. But through better knowledge of herself, and her microbiome, she's doing everything she can to beat the odds.

14.4 Colorectal Cancer

Paul was a pretty normal father of two teenagers when he noticed something odd in the bathroom. At first he thought it was something he'd been eating; despite a lifetime of Southern living, he didn't have as much tolerance for deep-fried cooking as some of his neighbors and the past few weeks had been unusually heavy on the grease. So, he laid off the french fries for a few weeks and it seemed to get better. He had enjoyed a lifetime of perfect health: he was rarely sick, had never been inside a hospital except to visit others, and fully expected to live well into his 80s or 90s like his grandparents.

He wasn't worried, but a few months later his wife reminded him that his company insurance plan includes a free annual physical, and he thought why not. The doctor didn't seem worried either, but suggested a few more tests "just in case", and unfortunately that's when he got the diagnosis that has been on his mind every day since: Stage IV colorectal cancer that has spread to his liver.

If you or a loved one find yourself in tragic situation like this, your first stop should always be with a medical professional. Paul's oncologist studied this full-time for years of medical school, has treated tens of thousands of cancer patients for 30 years, and gets paid to stay up-to-date on the latest science while interacting with other professionals like him. So it's beyond silly and arrogant – not to mention dangerous – to ask the opinion of an untrained amateur like me.

But still. Nobody, not even the most caring and selfless doctor, feels the urgency of the situation more than Paul and his family. They'll try anything; and who can blame them? The same careful, methodical and well-informed approach that makes the mainstream medical profession more effective over the long term, well, maybe it also makes this doctor just slightly more risk averse. There are treatments that no responsible doctor would consider, but what

[3] Pischel et al. (2014), available here: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5781629/#!po=75.0000

exactly is a "responsible" treatment when you know that your odds are tragically small? Seriously, what's there to lose?

Intriguing new discoveries have been made in the past few years about the relationship between cancer and the microbiome, and Paul asked if I know anything based on my years of near-daily sampling and amateur study. Might we find something in his microbiome, something that perhaps his doctor hasn't thought to consider? Given all that's known about microbiome-healthy diets, maybe if we found something unusual, some out-of-place microbe, is there a chance we might uncover a new, more effective treatment?

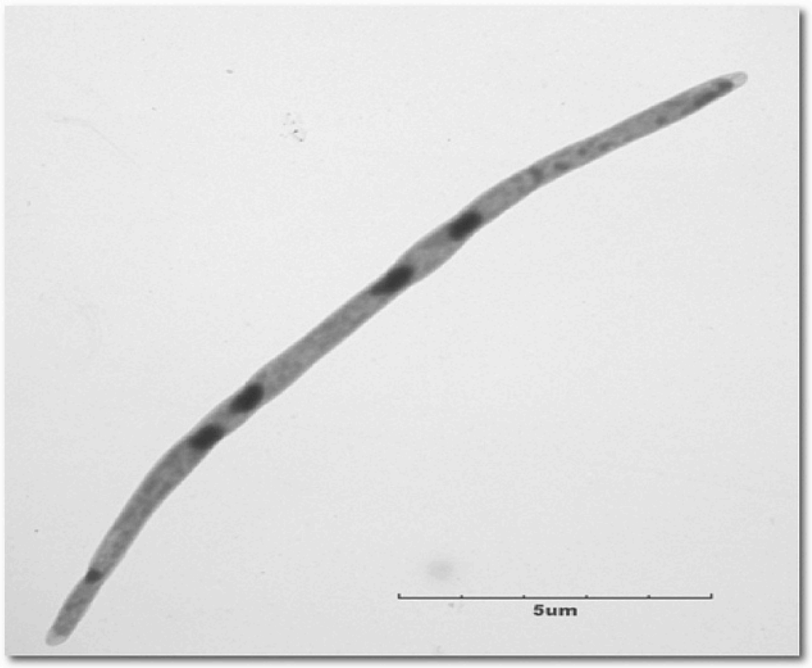


Figure 14.4: The culprit? Courtesy of Dr. Allen-Vercoe, University of Guelph Katz Lab

Many cancers seem to have a relationship with the microbiome. There are at least ten viruses which are known to be carcinogenic, including Human papillomavirus (HPV) that causes cervical cancer, and for which there is a vaccine. Some scientists guess that most cancers will eventually be shown to have their origins in a microbe, and although that's mostly speculation at this point, the idea of using microbes to treat or prevent cancer has attracted interest for more than 100 years.

Fusobacterium nucleatum, *Bacteroides fragilis*, and many members of the large class of Enterobacteriaceae have well-studied characteristics that make them liable to cause the types of genetic damage that can give rise to cancer.

I repeat: I'm not an expert – I have no training or credentials in this at all – so please don't take any of the following analysis as a substitute for the advice of a trained professional. Over the years, I've seen thousands of results from microbiome tests, including hundreds from people who claim to be healthy. We know for certain that Paul's chances are uncertain – even with the best medical help in the world. Who can blame him for reaching out to anyone else who might have some insights? The obvious place to start is to look at how Paul's microbiome sample may or may not differ from those healthy users.

Overall, I found his gut diversity is a little on the low side – a Shannon value of 1.2. I'm usually somewhere between 2.0 and 2.5, though it's not uncommon to see lower, and there's so much day-to-day variability in gut diversity that I wouldn't take a single result very seriously.

Next I looked at the broadest, Phylum level, where I ranked all the microbes in comparison to the healthy samples, picking out the top ones that seem to be outliers. The following charts show the percentage abundance of Paul's top microbes (yellow dots), compared to the average (blue dot) and median (red) for my database of healthy people. The vertical lines show the range of abundances I've seen in healthy people, so a yellow dot above or below that line is an outlier that's worth considering further.

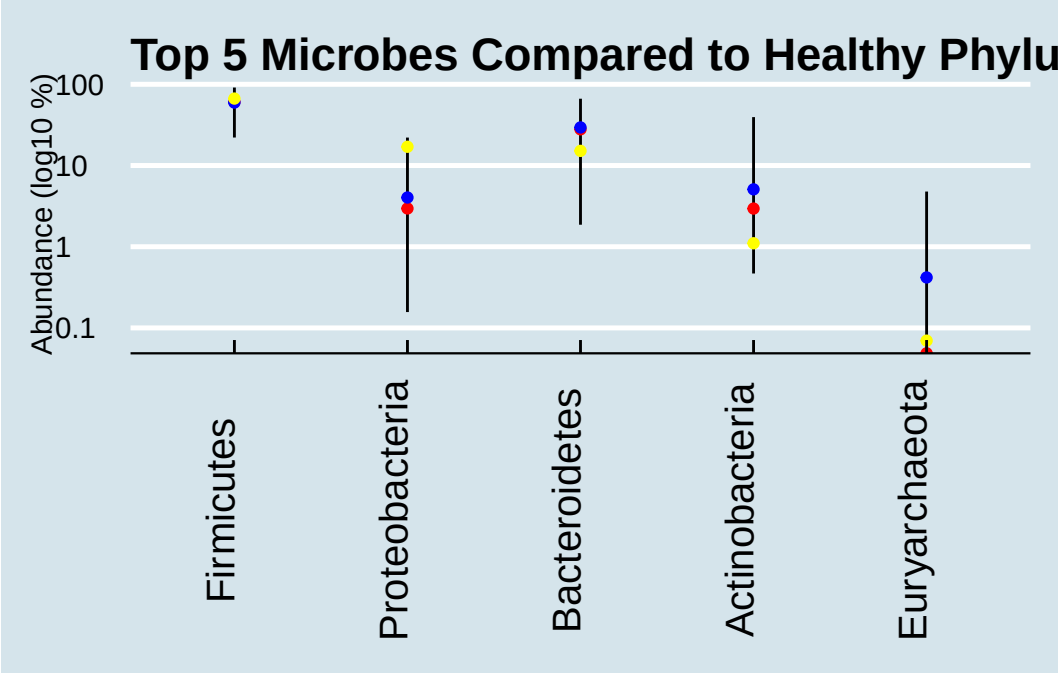


Figure 14.5: Healthy ranges compared (Phylum).

That high level of *Proteobacteria* is a clue that something's not right. I've noticed that this microbe tends to be high in people with gut issues; *Kluyvera*, *E. Coli*, *Shigella* and most common pathogens are in this group. Now, this is only one test, and it's not uncommon even in healthy people for the levels to show up high now and then. In my daily sampling, I've often had several results that high, including one or two at 25% for no apparent reason. Still, maybe it's worth looking more closely, at the Order level:

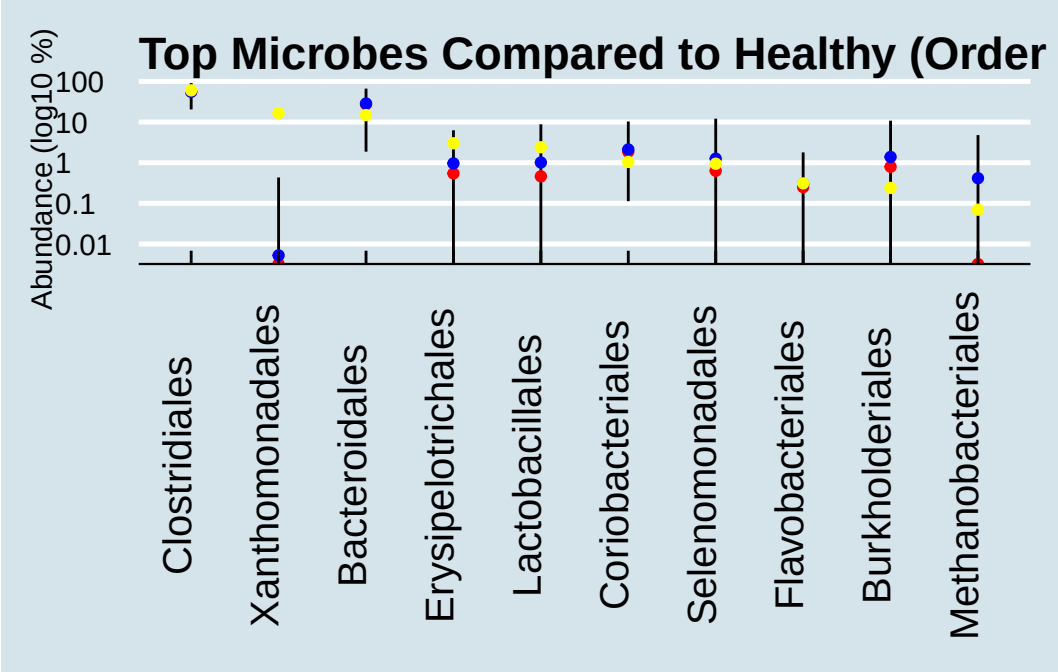


Figure 14.6: Healthy ranges compared (Order).

Here we see way, way off-the-charts high levels of the order *Xanthomonadales*. These microbes take up 16% of his entire gut microbiome! Of the thousands of microbiome results I've studied, I see this bacterium from time to time, but never at such high abundances. Other than Paul, the most I've ever seen in my own gut came after returning from my 2-week camping trip in New Mexico, where my total was 0.0056[4]. Paul's is 2800x that amount.

Not sure what this means, but one version of that bacterium is a pathogen that lives in things like catheters; it's usually harmless and goes away when you take out the catheter. Is Paul's chemotherapy "port" involved?

Let's look deeper, at the Genus level:

[4] I *did* see a level of more than 30% in one of my *skin* samples, again for no apparent reason (See more analysis).

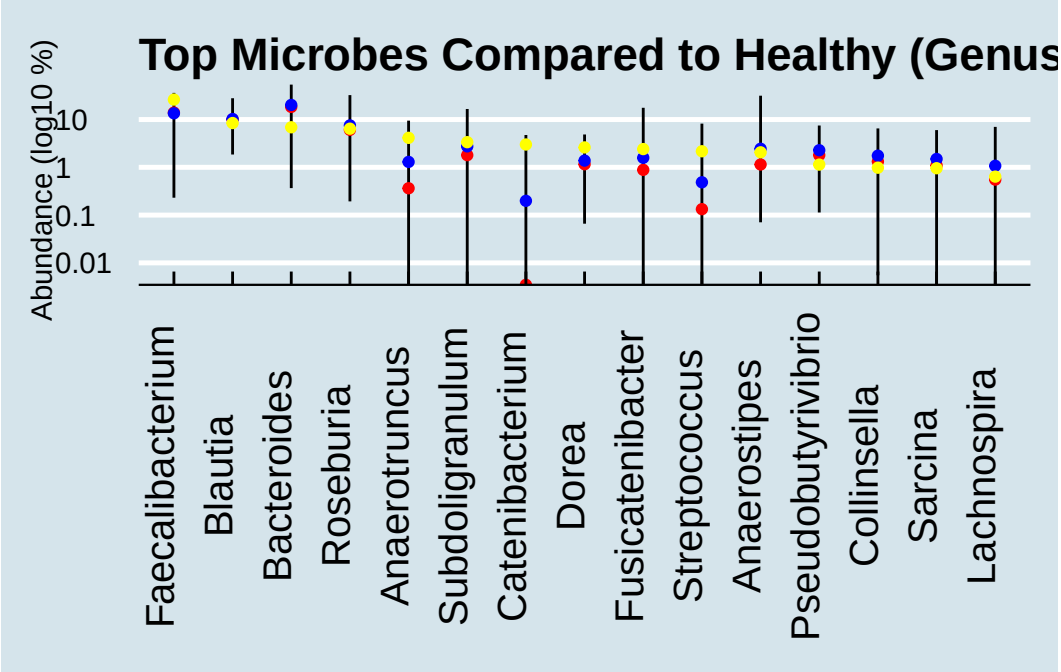


Figure 14.7: Healthy ranges compared (Genus).

Here the one notable outlier is *Catenibacterium*. Intriguingly, the other people I've seen with such high levels are all unhealthy. One of them, like Paul, is undergoing chemotherapy. Is this a microbe that associates somehow with disease? And if so, is there anything he can (or should) do about it?

Ken Lassessen at https://cfsremission.com has compiled an extensive list of actions that can increase or reduce common microbes and he finds evidence that flaxseed oil is associated with reduced *Catenibacterium*. Is it worth trying? Ask your doctor.

Peer reviewed studies of colorectal cancer and the microbiome have singled out *Fusobacterium*. In fact, that microbe is so clearly associated that destroying it with the antibiotic metronidazole slows tumors in mice. There is none in Paul's sample, either because his current cancer treatments have eliminated it, or because it just wasn't detected in this sample.

New research shows that, at least in people with an inherited gene known to pre-dispose the likelihood of colorectal cancer, the gut microbiome can form a biofilm composed of slightly-mutated versions of two microbes: *Bacteroides fragilis* and *Escherichia coli*.[5], neither of which is identifiable in Paul's sample.

[5]As reported in the Feb 1 2018 edition of the New York Times based on research published in Science: Dejea et al. (2018)

If you found some in your microbiome sample results, would that that mean you are at risk? The short answer is no. For what it's worth, among the hundreds of samples people have sent me, *B. fragilis* ranges between zero and 4% in healthy people, and zero to 7% in unhealthy people. Pretty inconclusive at best; misleading and counter-productive at worst.

Researchers have found many other intriguing links between specific microbes and colorectal cancer, including a recent study hinting at an association with the *oral* microbiome: low abundance of *Lachnospiraceae* in the gut apparently allows some oral pathogens to get a foothold in the gut mucosa.[6]

Another study (Jacouton et al. (2017)) found that drug-induced colorectal cancer in mice could be prevented by feeding them a probiotic strain of *Lactobacillus casei BL23*. Although the research is unlikely to apply to humans, this particular strain is known to affect the immune system, producing a cascade of molecules that appear to change a rat's response to cancer cells. For what it's worth, Paul's sample includes a bit of genus *Lactobacillus* and some *Bifidobacterium* too, though the test can't tell the particular strain.

Are any of these microbial connections worth further investigation? Sadly, the chances are slim, but keep in mind that the best scientists on earth don't know the answers either. It's arrogant and patronizing to suggest that patients and their families should defer only to "real" scientists on these questions.

We need all the personal scientists we can get.

See more references about the links between the microbiome and cancer, two good places to start are: Garrett (2015) and Fulbright, Ellermann, and Arthur (2017)]

14.5 Ketogenic Diet

THIS IS A VERY EARLY DRAFT

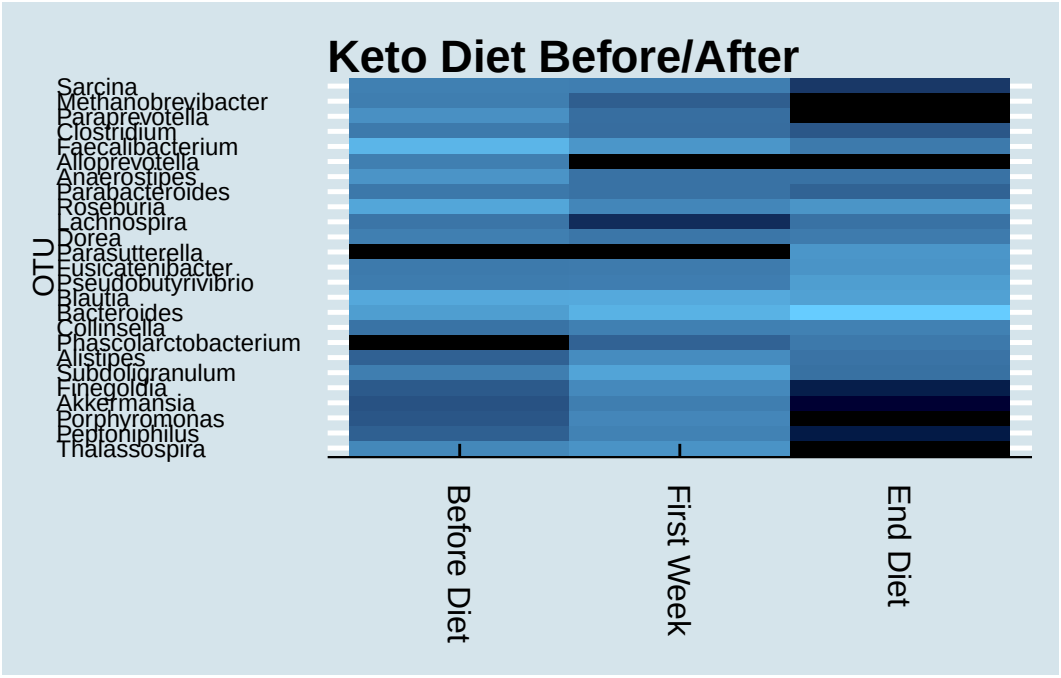
What happens if you eat a very low-carb, high fat diet ?

One study finds higher levels of *Akkermansia* and *Parabacteroides* reduce production of gamma-glutamylation and modulate hippocampal GABA/glutamate ratios.[7]

Here is the microbiome of a person who tried Keto for several weeks.

[6] see "Low colonic abundance of *Lachnospiraceae* favours colonisation of gut mucosa by oral pathogens linked to CRC" Flemer et al. (2017)

[7] Olson et al. (2018)



Now let's compare two people. The red line is the person above, who tracked their microbiome while beginning the diet; the blue line is a person who has been on a ketogenic diet for six months or so. Interestingly, the genus *Parasutterella* seems in unusually high abundance in both cases. Does it somehow relate to a low carb diet?

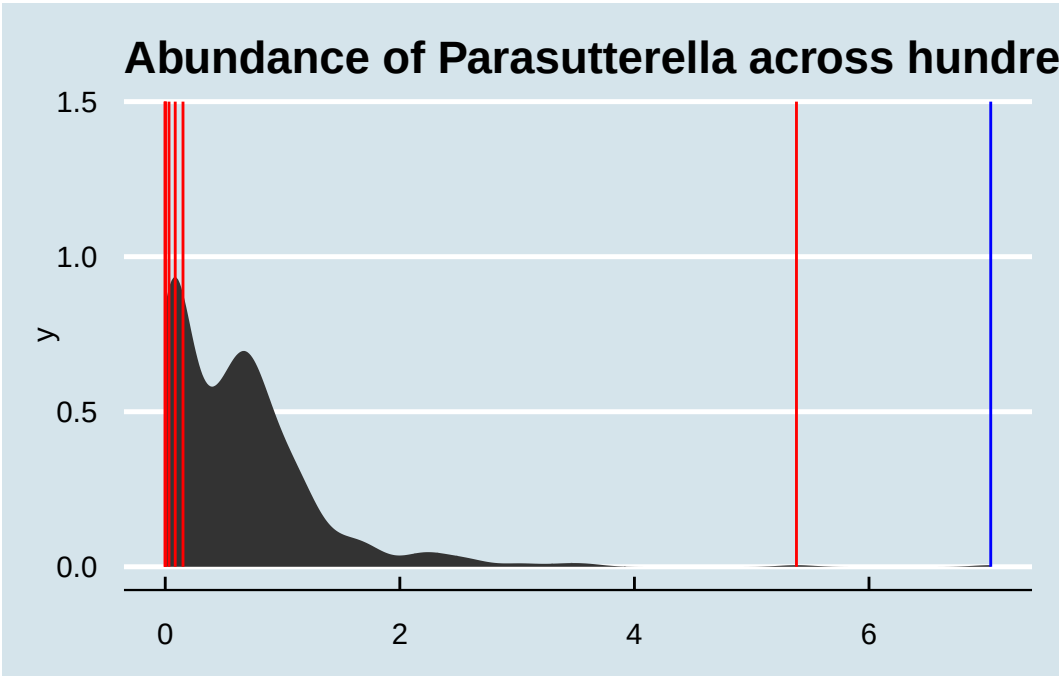


Table 14.3

	%
Bacteroides	37.90
Roseburia	9.84
Blautia	8.86
Pseudobutyrivibrio	7.43
Alistipes	7.18
Parabacteroides	3.89
Akkermansia	2.93
Lachnospira	2.64
Dorea	1.95
Clostridium	1.78

Microbes (genus-level) in a Parkinsons patient.

14.6 Parkinson's Disease

THIS IS AN UNEDITED EARLY DRAFT. PLEASE DON'T RELY ON IT.

Parkinson's disease is a devastating neurodegenerative disease that affects one in 100 people over age 60,

Although there is some evidence for a genetic component[8], environment clearly plays a role as well – which of course, may point to microbes.

A 2017 review finds this:

> Since 2015, six studies examining the gut microbiome in Parkinson's disease (PD) have reported an increase in Akkermansia abundance in PD patients (e.g., Heintz-Buschart et al., 2017; Hill-Burns et al., 2017); indeed, elevated Akkermansia abundance appears to be the most consistently defining feature of the PD microbiome. Likewise, a 2017 study found elevated Akkermansia in individuals with rapid eye movement sleep behavior disorder, which is considered a pre-motor symptom of PD (Heintz-Buschart et al., 2017).

Is this observable in our samples?

"Patrick" is a confirmed Parkinson's patient who sent me his microbiome test results. Let's look first at the genus overall picture Table 14.3

How different is Patrick's Akkermansia compared to everyone else? Figure 14.8

[8]Check out your LRKK2 status at 23andme

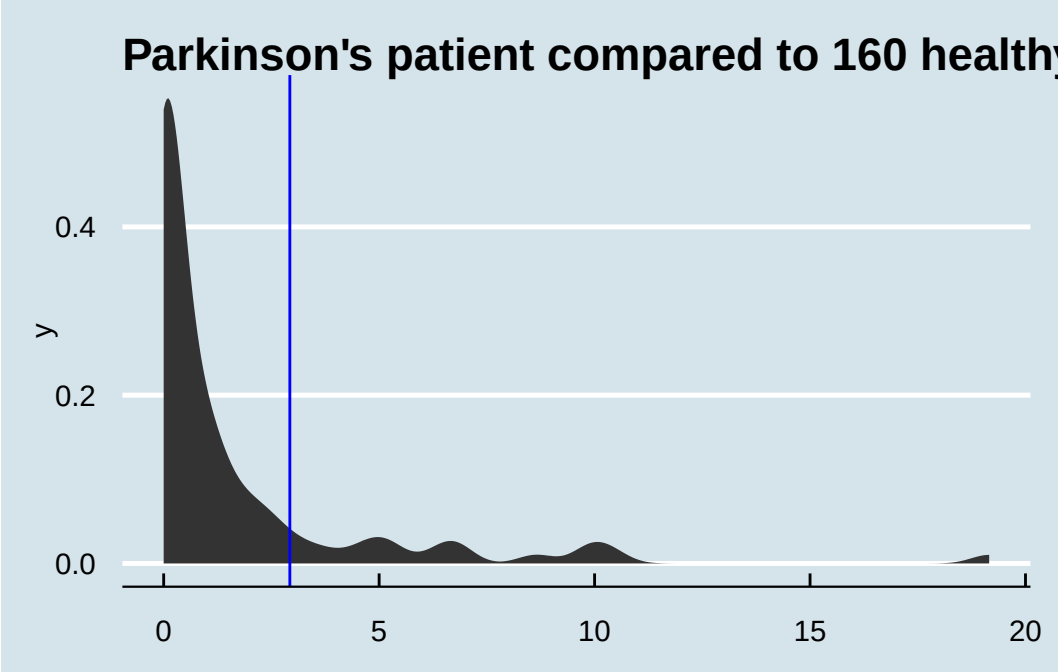


Figure 14.8: Density plot comparing Parkinsons patient to healthy users

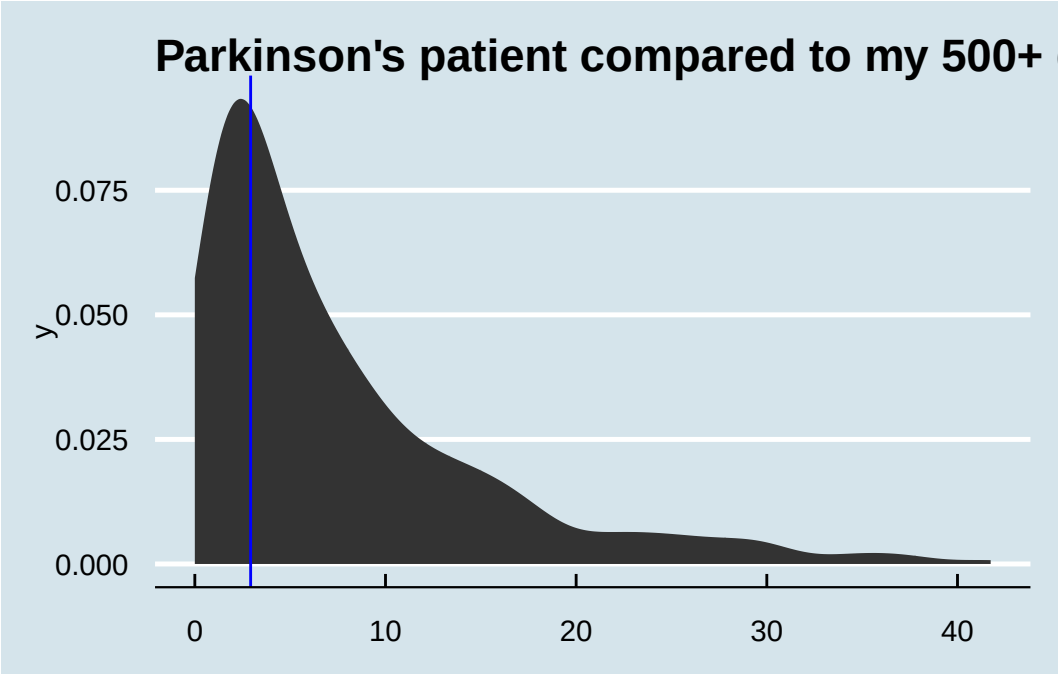
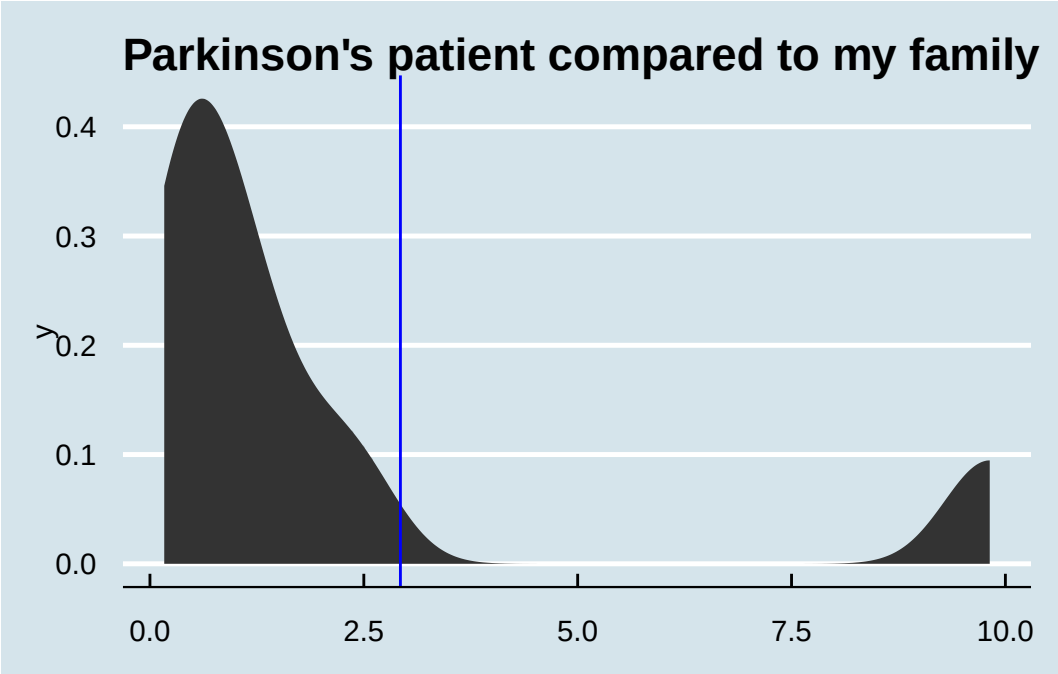


Figure 14.9: Density plot comparing Parkinson's patient to me.

How about me compared to my father and sister? (My brother, who I also tested, shows abundance of zero)



There are only 8 few samples involved here, and that one on the far right is just one of two from my sister and is therefore likely an anomaly. Still, if there were a big family component to this microbe, it certainly isn't showing in this test. F

Let's check the variability of my *Akkermansia*:

```
       0%        25%        50%        75%       100%
 0.000000   1.843725   4.010650   7.229650 37.161400
```

As you can see, my levels are consistently quite high, and sometimes *extremely* high.

A May 2019 study of 62 million electronic health records showed a slight increase in Parkinsons among people who had appendectomies[9], but smaller studies showed a slightly *lower* risk.

Hard to say…

[9] See https://www.cleveland.com/news/2019/05/link-between-appendectomy-and-parkinsons-disease-is-possible-cleveland-study-shows.html

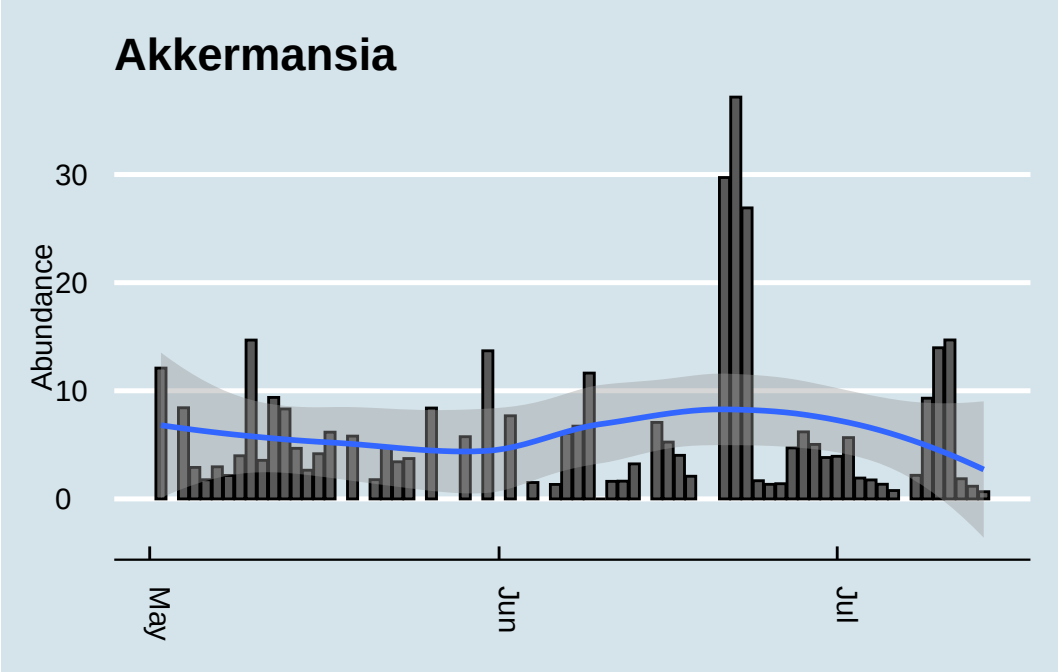


Figure 14.10: Quantifiying the variability of my Akkermansia

14.7 Autism

What can we learn from the microbiome of a 4-year-old boy with Autism? A mother sent me the Explorer results of her 4-year-old, who suffers from Autistic Spectrum Disorder (ASD)

Here's the overall picture of the phlyum-level microbes in his gut:

	ASD
Firmicutes	66.42
Bacteroidetes	27.68
Actinobacteria	3.00
Proteobacteria	2.89
Verrucomicrobia	0.01
Euryarchaeota	0.00
Fibrobacteres	0.00
Lentisphaerae	0.00

Looks quite normal, especially for somebody on an omnivore diet. Lots of Firmicutes and reasonable Actinobacteria. Proteobacteria is a smidge high, though not unusual for a single sample.

Let's look at more details, down to the genus level, and compare him to some similarly-aged (healthy) kids:

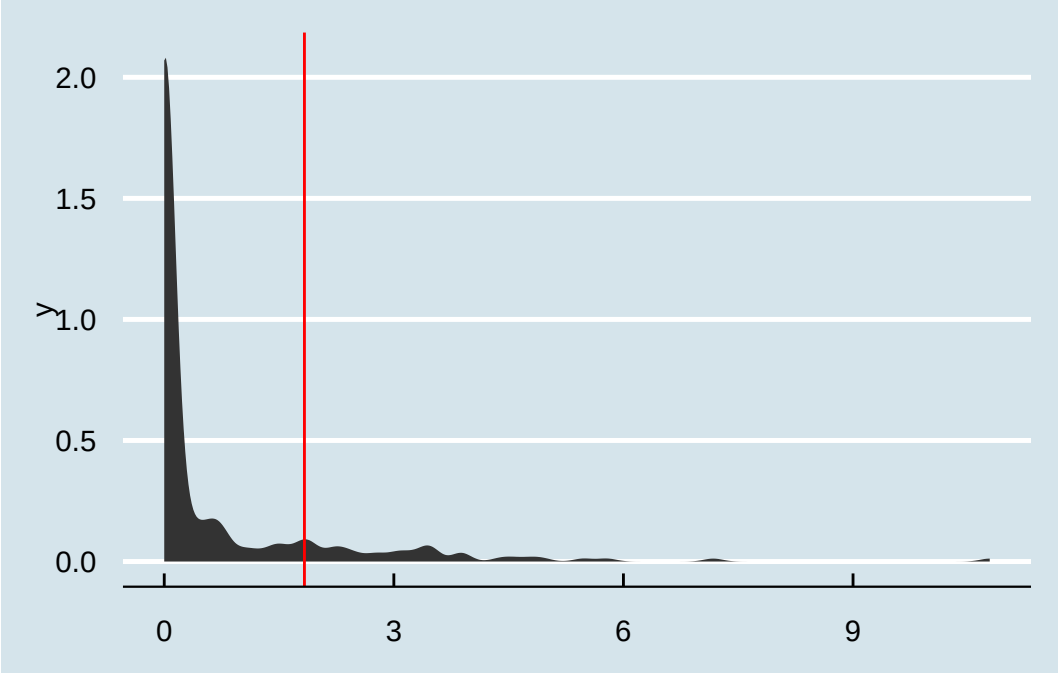
	Girl	ASD	Boy
Bacteroides	46.31	26.84	37.78
Blautia	8.12	13.84	10.81
Faecalibacterium	1.56	13.22	20.23
Roseburia	3.28	11.38	1.60
Anaerotruncus	0.94	6.44	0.21
Pseudobutyrivibrio	1.57	3.22	0.30
Subdoligranulum	0.48	2.80	3.36
Lachnospira	0.44	2.30	0.10
Collinsella	0.36	2.10	0.98
Anaerostipes	1.15	1.91	4.02
Sutterella	0.07	1.83	0.00
Dorea	2.24	1.42	1.59
Clostridium	0.08	0.86	0.51
Bifidobacterium	0.61	0.79	0.00
Parasutterella	0.00	0.44	1.70
Alistipes	5.26	0.34	4.60
Fusicatenibacter	1.40	0.34	0.00
Flavobacterium	0.51	0.34	0.00
Phascolarctobacterium	2.32	0.29	0.79
Flavonifractor	2.48	0.11	0.16
Thalassospira	2.43	0.07	0.00
Sarcina	2.97	0.04	0.00
Akkermansia	2.88	0.01	0.00
Barnesiella	2.34	0.00	1.92
Streptococcus	0.01	0.00	0.54

Now, what about the autistic boy is unique? Let's compare him to a similar-aged, healthy boy and see which microbes are present in the autistic case but *not* the healthy case:

	ASD
Sutterella	1.83
Bifidobacterium	0.79
Fusicatenibacter	0.34
Flavobacterium	0.34
Odoribacter	0.15
Asaccharospora	0.14
Thalassospira	0.07
Slackia	0.06
Aggregatibacter	0.05
Sarcina	0.04
Eisenbergiella	0.03
Lactobacillus	0.03
Akkermansia	0.01
Finegoldia	0.01
Prevotella	0.01
Corynebacterium	0.01
Neisseria	0.01

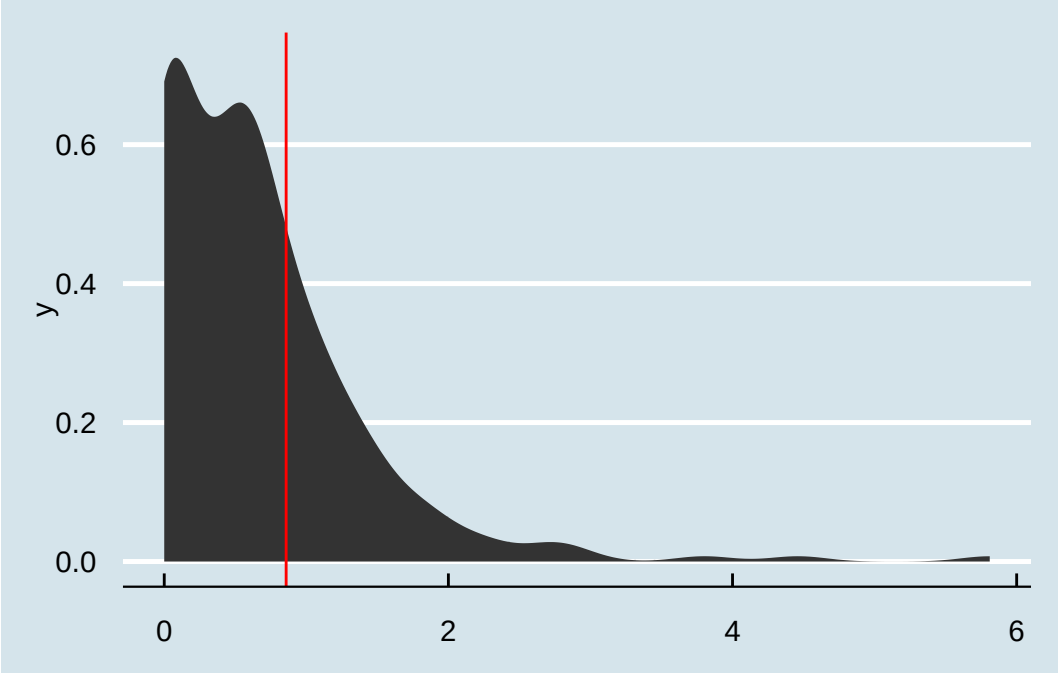
The autistic boy has *Sutterella*, an organism that is missing in the healthy boy.

How unusual is that level of *Sutterella*? I don't have enough samples of healthy boys to do a real comparison, but just to get a rough idea of what we're dealing with, here's the range over a mix of 100+ health and unhealthy samples (mostly adults). The red line indicates where the autistic boy fits on the range of *Sutterella* abundances:



14.7.0.1 Microbes of known association with autism

Clostridium tetani may play a role in autism [citation needed], though unfortunately our 16S test can't see this microbe. Instead let's look for that microbe at the genus level:



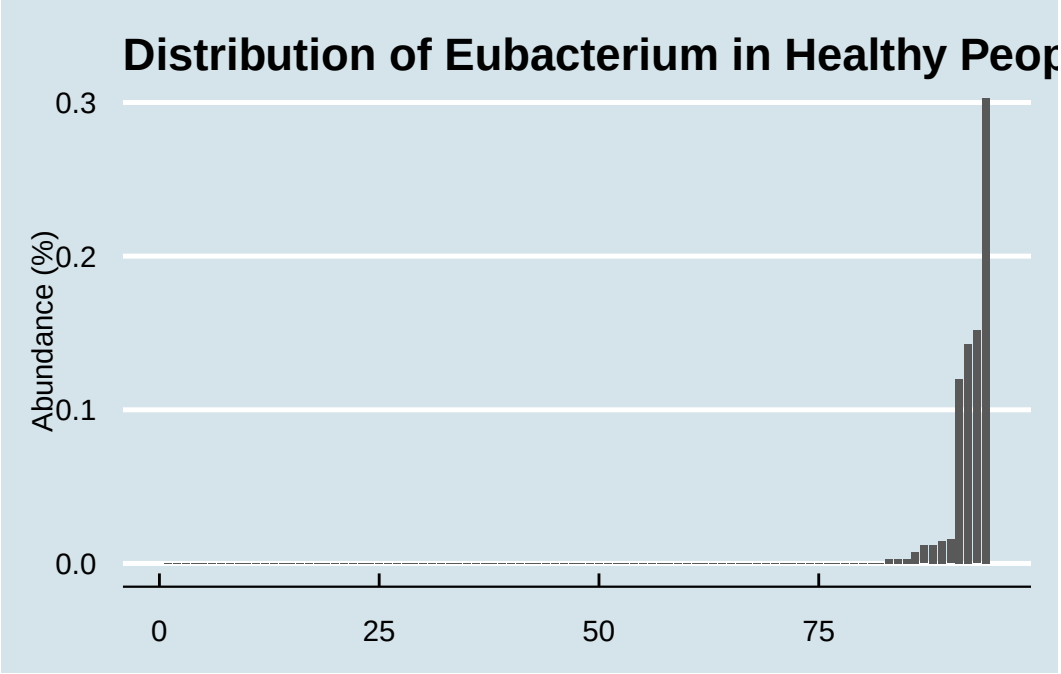
Although obviously a single test can't say much, he is not especially unusual for this microbe, at least at the genus level.

14.8 Exercise

I wanted to see if this paper is right in concluding that physically-fit women have lower *Eubacterium*. Here's one physically-fit woman I know and sure enough, she has none of this microbe.

Other people I've seen who *do* have this seem to be either (1) older, or (2) on an unusual diet.

Here's a summary of the ranges I see in healthy people


Distribution of Eubacterium in Healthy Peop
Abundance (%)
0.3
0.2
0.1
0.0
0
25
50
75

	Nov8	diarrhea
Faecalibacterium	132088	159757
Bacteroides	124273	138547
Blautia	68357	151024
Asteroleplasma	55081	15192
Dialister	44186	12737
Roseburia	39761	66889
Sarcina	34326	7123
Collinsella	32053	18070
Fusicatenibacter	26337	10282
Subdoligranulum	24548	32277
Sutterella	24507	71739
Catenibacterium	24426	9578
Lachnospira	22221	4105
Anaerostipes	22207	24691
Alistipes	21965	17688
Dorea	21091	29480
Bifidobacterium	17862	1287
Anaerotruncus	16854	1650
Thalassospira	14553	442
Parabacteroides	13545	11731
Akkermansia	13531	8914
Pseudobutyrivibrio	11487	27910
Clostridium	11137	12858
Flavonifractor	10451	1147
Flavobacterium	5420	5956

Although these people are all healthy, it's possible that those with *Eubacterium* are more active than those without. That's an investigation for another day.

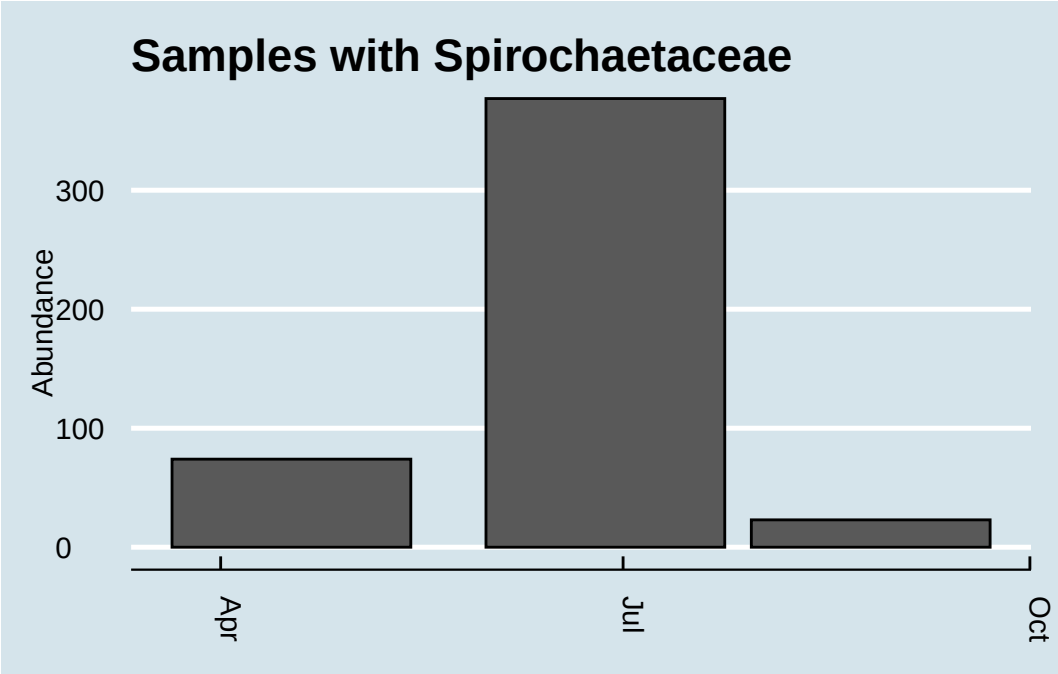
14.9 Lyme Disease

NOTE: THIS CHAPTER IS AN EARLY DRAFT

What can we learn about Lyme disease?

We know that Lyme disease has been linked to the pathogen *Borrelia.* The uBiome 16S pipeline doesn't appear able to distinguish among the different species of that taxa, so let's look at a higher level, the family *Spirochaetaceae*, which includes *Borrelia.* Several people with confirmed lyme disease sent me their samples, and indeed I *do* find some *Spirochaetaceae* in these samples, albeit at very low abundance.

I was unable to find any *Spirochaetaceae* in any of the other hundreds of samples I examined, including from a few people with confirmed lyme.



Let's do an ordination. How do lyme patients resemble one another? Figure 14.11

This person tried Kefir to see if they could increase Fusicatenibacter:

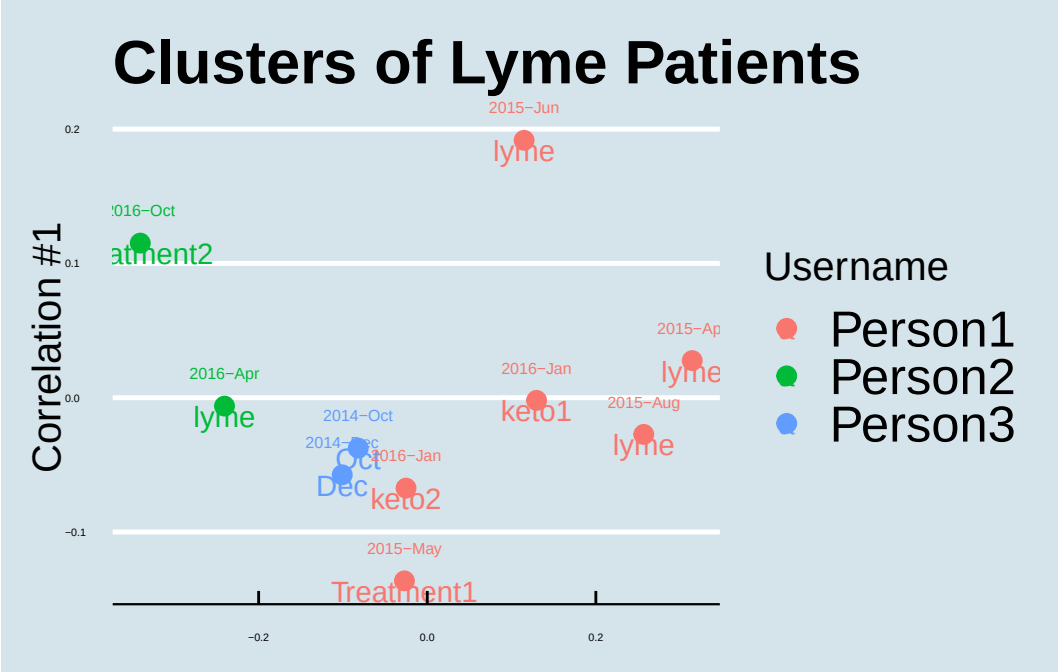
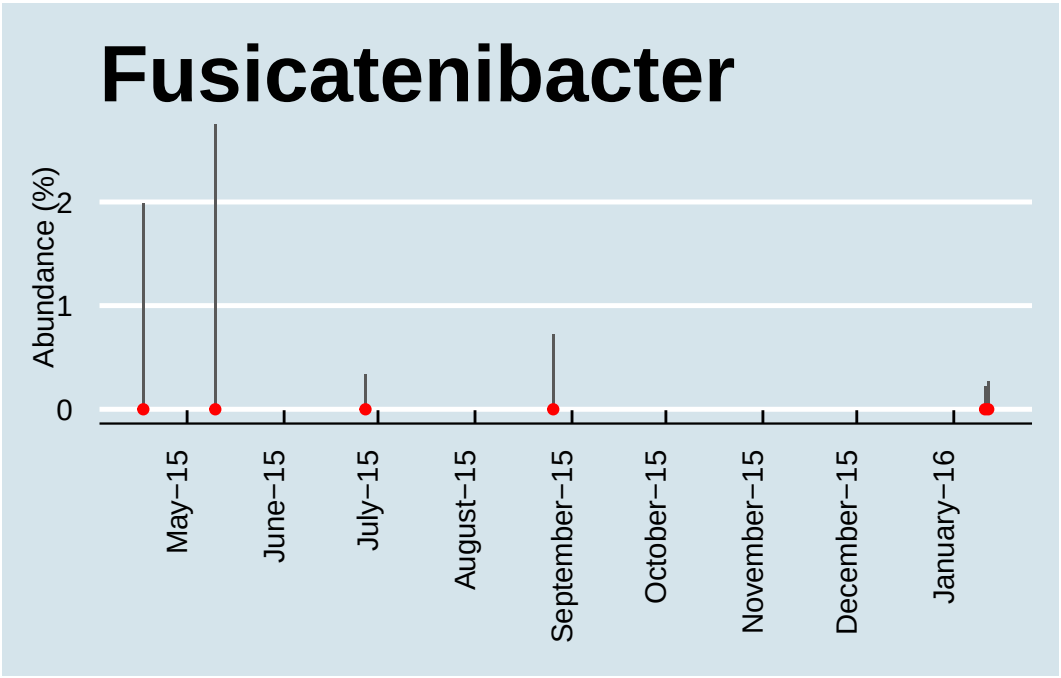
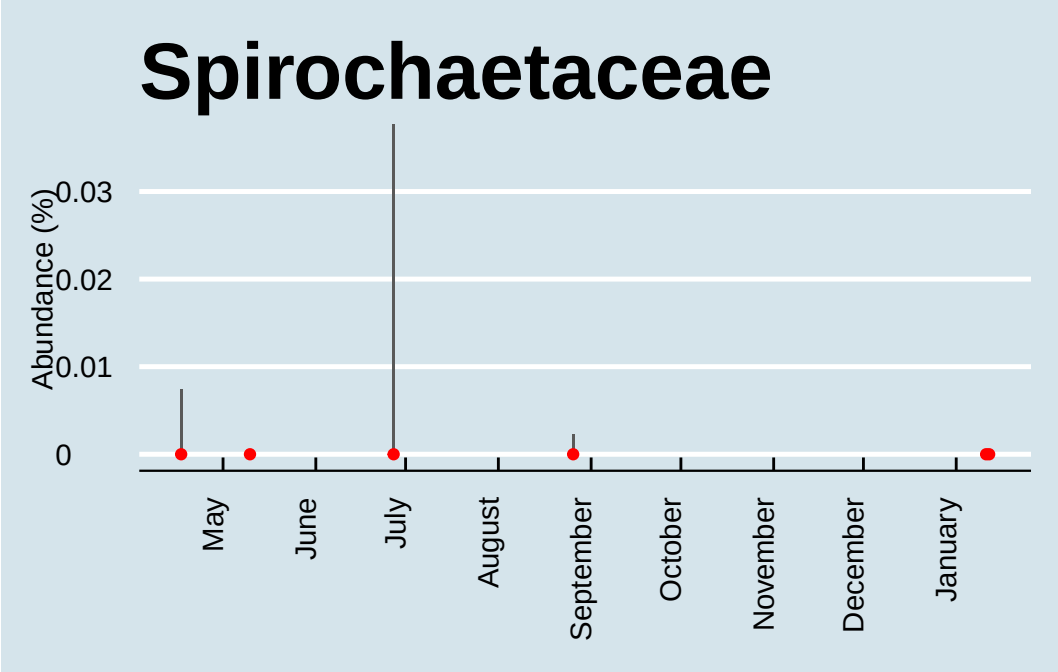


Figure 14.11: Clusters of lyme patients




Spirochaetaceae
Abundance (%)
0.03
0.02
0.01
0
May
June
July
August
September
October
November
December
January

15 DIY Microbiome Testing

Update May 2024: Ken Lassesen's list of DTC microbiome tests:

Which tests are best to use ranks tests based on how easily they let users extract data. For US users, he recommends Thorne Labs and Ombre Labs. Most of the high-resolution tests will cost about $200.

Although I've done hundreds of near-daily microbiome tests in the past, these days I only test myself every couple of months. Here are my recommendations as of early 2023.

If you're just curious to see the results, I recommend signing up for one of the free clinical studies that will send you a gut kit and report the results:

- The MACO study from Endominance wants to understand the relationship between the microbiome and anxiety. Fill out a 100-question survey to get a gut test kit. They'll pay you $40 and give you your results when you're done.
- NYUFamili gives you a $25 gift card for completing a questionnaire and emailing a gut sample.

If you're more serious and want to get a detailed breakdown of the microbes, I recommend either:

- Ombre, which for $100 will give you a very broad ("16S") look at your gut microbiome.
- Tiny Health ($200), which though specializing in infant and women's health, offers a good general-purpose high-quality report for adults as well.

Figure 15.1: A few good microbiome tests from 2023

Many people are looking for a test that will help them with a diet, either to lose weight or to solve some other gut-related issue. In that case, I would look at:

- Zoe ($250), which includes a very readable report, plus an app that helps you target specific foods and amounts. (See my detailed review)
- Viome ($300), including a blood and saliva test based on their "transcriptomics" technology. You'll get a ton of information, mostly related to food suggestions.

If you're suffering from a specific ailment that you'd like to consider for a microbiome-related treatment, I strongly suggest you see an expert. Search your local area for "digestive" or "gut" doctor, or for a condition like "IBS" or "SIBO". Go with doctors who use tests from Doctors Data or GI MAP from Diagnostic Solutions. Unfortunately there is wide variation in quality among gut doctors, so you'll need to shop around, and hopefully get a referral from somebody you trust.

A University of Oslo dietician at MyMicrobiome did a thorough feature summary of microbiome tests available in Europe. While not all of the tests are available in the US, it's a good breakdown of what to look for in these tests.

Part V

Next Steps

16 Beyond Bacteria

While technically the microbiome refers to all microbes in and around us, most of the everyday usage of that term is limited to bacteria. But bacteria aren't the only microbes in you, and it's possible that they aren't even the most important. There are fungi, of course, and perhaps other too-tiny-to-see lifeforms like protozoa, but one large class of microbes appears to have a major effect on us but is rarely studied: viruses.

Viruses present several problems for scientists. They're super-tiny for one: you can often fit hundreds of virus-sized particles inside a single bacterium. They're not always made of DNA, and even when they are, they don't reproduce on their own.The controversy about whether they should even be considered "alive" is partly due to this lack of reproduction ability, but also because many of them appear to be quite simple: just a sequence of proteins.

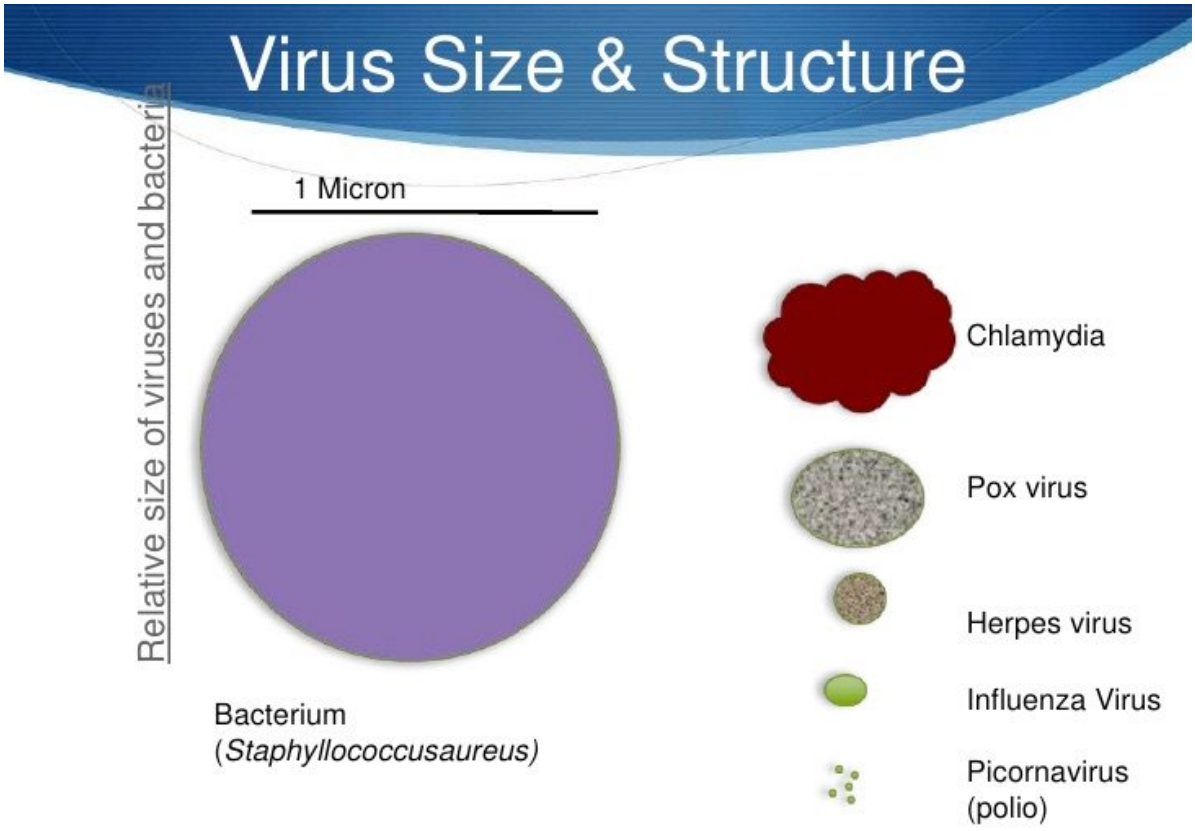


Figure 16.1: Viruses are tiny compared to bacteria. Image:marneechua

These characteristics wreak havoc with the traditional scientists' toolkit for dealing with small objects: they're too small to study optically without powerful equipment, and their lack of a reproductive mechanism means you can't easily amplify their quantity, and their RNA components are unstable and difficult to work with. It's so much easier to deal with bacteria.

But thanks to some ingenious and difficult work, a few things are clear.

Viruses, like bacteria, are everywhere. In fact, just about every human on earth is infected, right now, with dozens of them.[1]

[1]Virgin (2014)

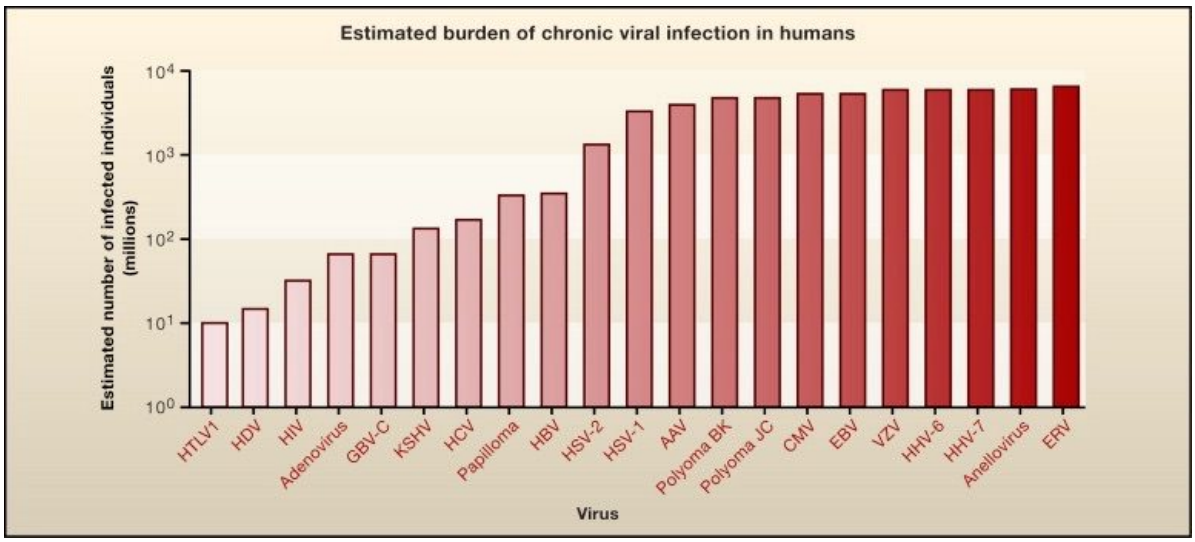


Figure 16.2: "You already have an infection with these viruses." Herbert Virgin at 2015 NIH Dyer Lecture

Unlike bacteria, viruses are so tiny that they can slip through cracks in the body that would normally stop bigger pathogens. The placenta, for example, can pass viruses like rubella (German Measles), cytomegalovirus, HIV, and Zika.

Some viruses may actually be good for us. The ratio of viruses to bacteria is higher on the body's mucosal surfaces, such as in the gut or the nose, perhaps because these viruses (called phages) are programmed to attack pathogenic bacteria before they make it through the mucosal lining.[2]

A virus that circulates in your body is considered "latent" if you appear to be none the worse for it, but it's hard to tell if that's ultimately good or bad. Humans have been studying viruses for only a few decades, but viruses have been studying *us* for, well, forever. The chromosomes of virtually every organism on earth shows the tell-tale signs of viral interference.

Latent viruses may not cause any obvious symptoms, but they continue to hijack cells, vigorously making copies of themselves, inhabiting every nook and cranny of the body while waiting for something to happen. In mice, and presumably humans, it's been shown that a particular type of listeria infection is inhibited when a formerly-latent virus gets word that the bacterium is in the body. The listeria pathogen ordinarily causes terrible disease symptoms, but in the presence of a latent herpes virus, nothing happens.

The nasty helminth worm, scourge of the tropics and an enemy of humans since time immemorial, actually wakes up the latent herpes virus, which has a sensor fine-tuned to detect it, in turn causing another reaction that will shut down the helminth again. If the virus is not

[2]Barr et al. (2013)

present, guess what: the worm goes about its awful parasitical business; but with the virus, nothing happens. So which is worse: herpes virus or a helminth infection? Best, apparently, is to have both. Trouble awaits the body who has only one or the other.

This is true all over evolution and it may explain why some studies are frustratingly hard to reproduce. An experiment that works in one lab, with the same type of animal with the same food, doesn't work in another lab, no matter how carefully they try to make the experiments identical. Maybe the only difference is that one location happens to have a geographically-specific virus lurking about, and that is just enough to activate a cascade of reactions that nullifies the experiment. What a pain.

Much popular microbiome advice suggests that *more* diversity is better, and intuitively it makes sense that a body with a variety of different microbes has a more robust defense system than somebody with a more restricted microbiome. Whether this is always true among bacteria is open for debate — I for one think it matters a lot *which* microbes you have, rather than the variety alone — but in the virome there is evidence that *more* diversity causes more problems.

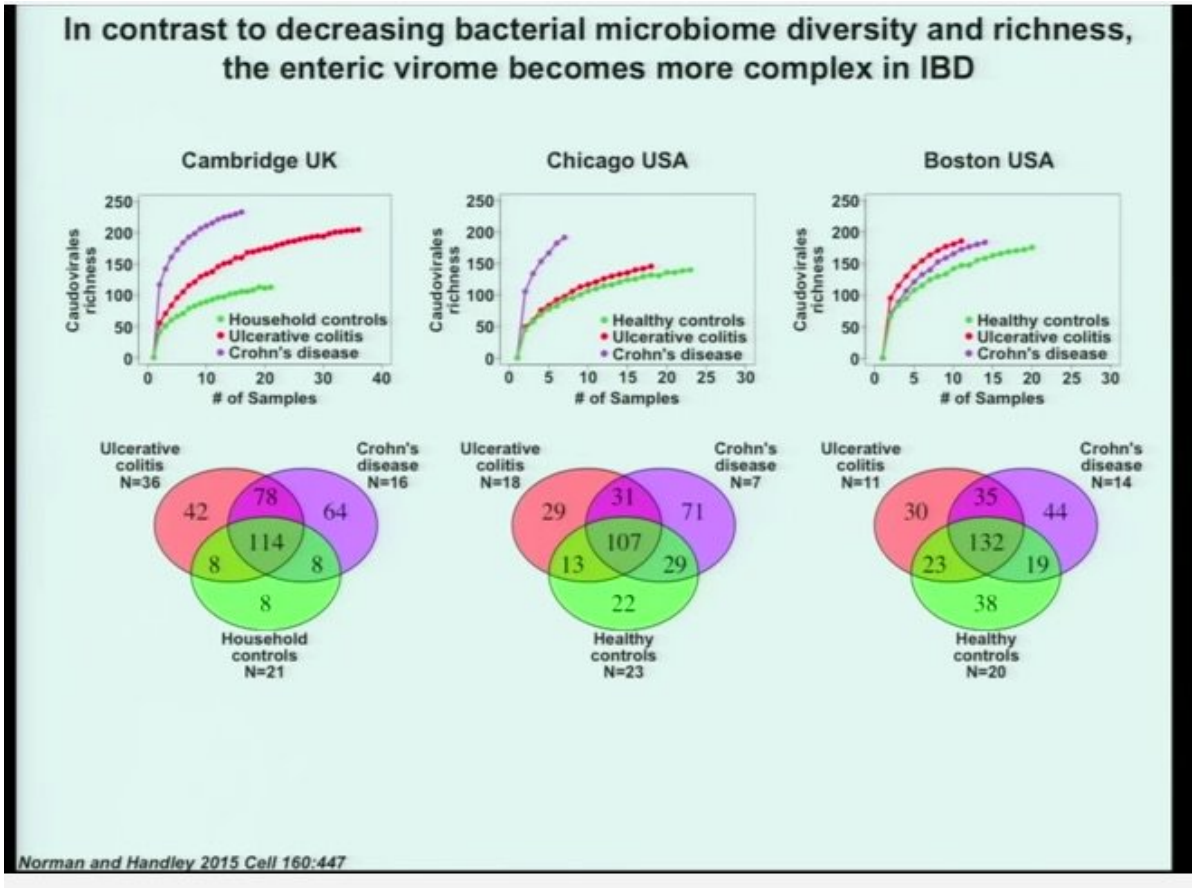


Figure 16.3: Increased diversity is not always good.

IBD and Crohn's patients who were carefully studied alongside healthy controls appear to have a wider variety of viruses in their systems. It's too early to say whether this has any implications for treatment, but it does point out that the story is more complicated than we'd hope.

Similarly, the virome of colorectal patients is so different from healthy people that researchers suspect the cascade of events leading to cancer may be triggered by differences in the way phages affect gut bacteria, and not the bacteria themselves. Furthermore, the phages seem to act as a community, making it unlikely that a single culprit starts the process. Rather the cancer results in some unknown, impossibly complex disturbance in the community as a whole.[3]

In fact, if you're not super careful to control for the variety and types of viruses present, it could be that taking a probiotic could be harmful. Your body may have a perfectly good

[3]Hannigan et al. (2018)

reason that a particular, otherwise beneficial microbe is missing or lower in quantity than in comparable healthy people. Introducing a bacterium that reacts with a latent virus could actually cause more harm than good.

There's no solid evidence for this yet, but if true, it points in the same disturbing direction for treatment that we've been afraid to admit all along: the best way to treat any disease is through ordinary food. Don't go messing with microbes unless you know what you're doing.

16.0.1 The gut phageome

About half of all people appear to share at least some of a core group of phages, leading some scientists to speculate that, beyond the microbiome may lay a "Healthy gut phageome" (HGP). Another group of phages seems to be much rarer, occuring generally in people of various disease states. Could it be that it's the *phages*, not the microbes themselves that drive some types of disease?[4]

16.0.2 Insects and the microbiome

(DRAFT)

see "The tiniest tiny genomes" 10.1146/annurev-micro-091213-112901

From Rachel Thomas

> An astonishing 60% of insects (including butterflies, bees, and beetles) around the world have the bacteria *Wolbachia*. This fascinating bacteria can have some surprising impacts, including reducing the ability of mosquitoes to carry or transmit dengue virus.

[4] see Manrique et al. (2016) full text)

17 Microbes and Genes

There is growing evidence that your human genes affect which microbes you'll host.[1] If you have your 23andme results, click through on the following links to see what your own genes are.

Secretor FUT2 allele

RS601338 If you are AA, then the good news is you are immune to norovirus but the bad news is you don't digest fiber efficiently, which obviously changes the types of microbes you'd collect. With more data, we'd figure out which types of fiber work and which don't in people like this. I know several people who are AA and have serious health issues — I'm convinced with this knowledge we'd just need to find the right (probably very weird) diet for them.

This gene correlates highly with *Bifidobacterium*.[2]

HLA-B27

RS6919835

If you have A, you're more predisposed to autoimmune conditions (like multiple sclerosis) but it's believed the inflammation itself is caused by *Klebsiella* bacterium, of which interestingly I'm one of the very few people who shows any in their uBiome results. So am I immune to MS? Or if I somehow transmit my *Klebsiella* to an A carrier, could I infect them with MS?

Caffeine

rs762551

I'm AC, which means I'm a slow metabolizer. 23andme thinks that I should stay away from coffee for that reason, but it's not true! I drink as much as I like with no effects on sleep, and meanwhile uBiome's functional KEGG test shows I'm 3x more efficient at caffeine metabolism than other people. Why? Because I must have a bug that does the work my genes don't. Finding that one would be pretty cool.

Lactose intolerance

rs4988235

I'm A/G, but people who have a T variant are likely to be lactose intolerant.

[1] For example see Lim et al. (2017)

[2] See publications by Pirjo Wacklin including: Wacklin et al. (2014) and Wacklin et al. (2011)

HNF4A: diabetes risk in Asians

rs4988235

This gene is associated in part with microbes.[3]

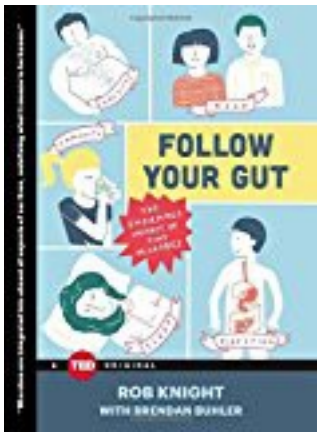
The website Genetic Life Hacks has another list of 23andme SNPS that relate to the microbiome.

[3]see this study: http://genome.cshlp.org/content/early/2017/05/16/gr.220111.116.abstract

18 My favorite books about the microbiome

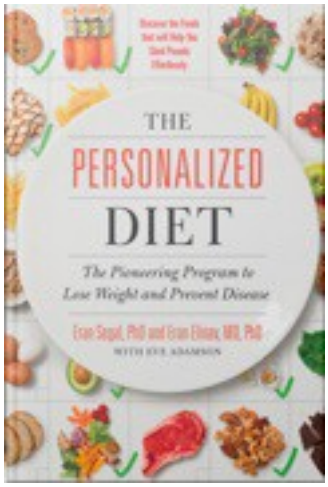
The number of books about the microbiome have exploded in the past few years, but I've tried to read (or at least skim) every book I can find. If you're a newcomer who would like to come up to speed, here's my ranked list of the best books as of today (early 2020).

- Knight, Rob *Follow Your Gut: How the Ecosystem in Your Gut Determines Your Health, Mood, and More*



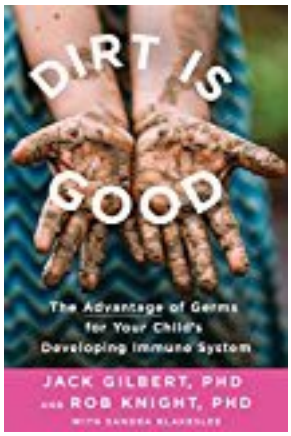
At only 120 pages, this is the most concise summary of what's known — and not known — about the microbiome. Written by one of the scientists behind the American Gut Project, it's a readable and fascinating overview of the facts and a great first introduction. If you only read one book, this is it.

- Segal, Eran *The Personalized Diet: The Pioneering Program to Lose Weight and Prevent Disease*



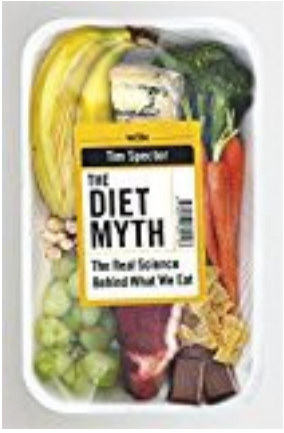
No two bodies respond to food the same way, and the scientists who discovered some important links to the microbiome have written the most actionable, microbiome-informed book I know about weight loss and diabetes prevention. Focusing on glucose response, they describe their most famous experiments in easy-to-read but well-informed scientific language. Learn why artificial sweeteners aren't good for you, why averages are a poor way to choose your diet, and simple tricks to measure precisely what will work for you.

- Gilbert, Jack *Dirt Is Good: The Advantage of Germs for your Child's Developing Immune System"*



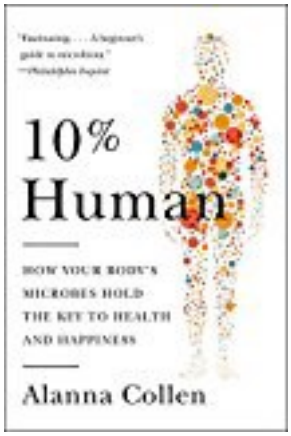
The most practical, up-to-date book on what works and doesn't work. Written for parents as a guide to ensure a child's microbiome is as healthy as possible, you'll find dozens of yes/no practical answers to everyday questions: "Should we get a dog?" (yes), "Are GMOs safe?" (yes), "What works for diaper rash?" (probiotic yogurt) and much more. Because, as the book points out, the microbiome changes little after about age three, most of the advice is general enough to apply to adults as well.

- Spector, Tim *The Diet Myth: Why the Secret to Health and Weight Loss is Already in Your Gut*



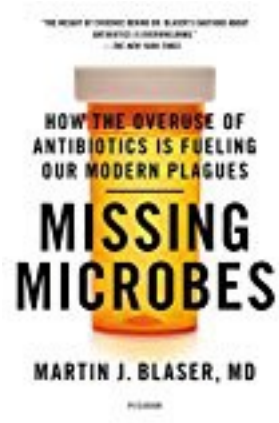
Another excellent one written by a practicing scientist and a good complement to *Follow Your Gut*. The author's concise writing style packs multiple interesting examples and facts on each page. Divided into chapters based on type of food, I learned about what's proven and what's unknown about the effects of different diets on health.

- Collen, Alanna *10% Human: How Your Body's Microbes Hold the Key to Health and Happiness*



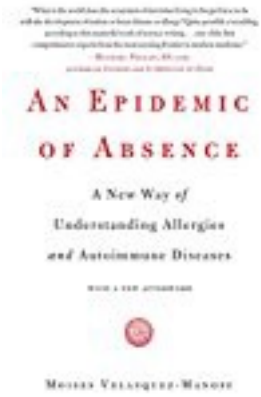
Another great overview that explains to the ordinary person the implications of the microbiome. Well-written, full of interesting facts, but sticks strictly to mainstream science. For example, although the author explains the concept of "leaky gut", she observes it skeptically as an unproven hypothesis, rather than jump whole-hog into diagnoses. Best parts discuss the gut role on behavior ("Mind Control"), with detailed examples from autism research, Whipple's Disease, and more. The book includes a list of the highest-quality references, but unfortunately it's not complete, so many of the facts are hard to follow up.

- Blaser, Martin *Missing Microbes: How the Overuse of Antibiotics Is Fueling Our Modern Plagues*



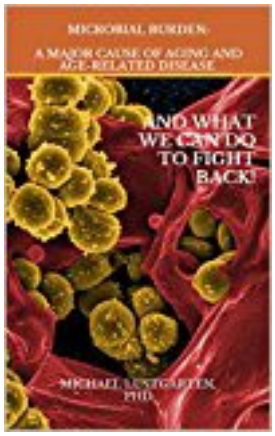
The author is a long-time, highly-cited microbe researcher who did much of the original work on *Helicobacter pylori*, the stomach bacterium implicated in ulcers. But studying these germs up close for so long has made Dr. Blaser much more nuanced about what constitutes "good" and "bad". Quoting the Inuit, "Wolves keep the caribou healthier", he makes the case that many modern ailments like allergies or diabetes maybe caused by the *lack* of microbes, not their presence.

- Velasquez-Manoff, Moises *An Epidemic of Absence: A New Way of Understanding Allergies and Autoimmune Diseases*



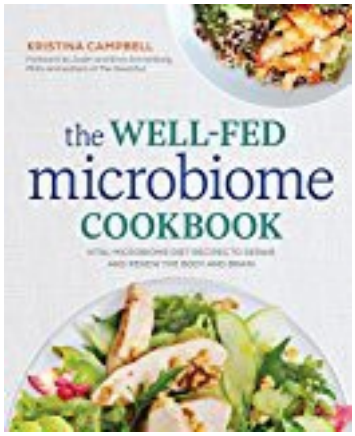
Another book that explains a provocative idea that our immune systems need regular stimulation by parasites and other infectious agents, or we risk unpleasant side effects in the form of allergies, diabetes, and many other nasty conditions. The remarkable correlation between the hygiene of modernity and the rise of autoimmune diseases makes for powerful evidence that science is far behind in understanding all the consequences of our current lifestyles.

- Lustgarten, Michael *Infectious Burden: The Cause Of Aging And Age-Related Disease*



18.1 Other Books Worth Having

- Campbell, Kristina *The Well-Fed Microbiome Cookbook*



If you'd like a shorter, summarized version of the science as well as practical suggestions for family meals, get this book. The author writes for gutmicrobiotaforhealth.com, which you should check for more up-to-date information.

- Axe, Josh *Eat Dirt*

I was surprised at how much new and practical information was packed into this book.Although the author is not a practicing scientist himself, he summarizes new ideas efficiently and I learned much about medicinal plants, non-Western treatments, and essential oils.

18.2 Other

Just about any new health book from the past few years will touch on the microbiome, but I didn't find much specifically microbiome-related that was useful or new in books by David Asprey ("The Bulletproof Diet"), Tim Ferriss' *Four Hour Body*, or Chris Kresser's *Paleo Code*. You may find these books useful for other reasons, like the detailed discussions of specific foods, but you'll learn little about the microbiome.

19 Best Academic Papers

If you're new to the microbiome and would like to dig into the academic papers that drive the field, here's the selection that I consider required reading.

Microbiome science is in its infancy, but its enormous potential makes it an environment rich in highly speculative research, often with results that are overturned rapidly with new discoveries. So before you read anything else, I encourage a peek at this 2014 Nature article by Harvard epidemiology professor William P. Hanage: Microbiome science needs a healthy dose of skepticism

19.0.1 Popular Topics

You will find several themes repeated regularly in the popular microbiome press

19.0.1.1 The Firmicutes/Bacteroidetes ratio

The most recent, well-respected review Walters, Xu, and Knight (2014) Walters, Xu, Knight 2014 says flat-out:

> the ratio changes between normal and obese individuals are not statistically significant overall and therefore should not be considered a general feature distinguishing normal and obese human gut microbiota across populations.

Another study (Finucane 2014) goes into deeper statistical detail to conclude the same thing.

Walters, Xu, and Knight (2014)

Walters William A.,Xu Zech and Knight Rob(2014), Meta-analyses of human gut microbes associated with obesity and IBD, FEBS Letters, 588, doi: 10.1016/j.febslet.2014.09.039

Finucane MM, Sharpton TJ, Laurent TJ, Pollard KS (2014) A Taxonomic Signature of Obesity in the Microbiome? Getting to the Guts of the Matter. PLoS ONE 9(1): e84689. doi: 10.1371/journal.pone.0084689

19.0.1.2 Obesity and the microbiome

Although it's exciting to think that an obesity cure might be found in the microbiome, the most recent reviews shows that it's more difficult than originally thought. Here's the best summary Full Text (open)

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@sze_looking_2016 Sze, Marc A., and Patrick D. Schloss. "Looking for a Signal in the Noise: I
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19.0.1.3 "We are only 10% human"

It's a number based on a guess dating from 1977, but finally updated in 2016:

> Our analysis updates the widely-cited 10:1 ratio, showing that the number of bacteria in our bodies is actually of the same order as the number of human cells. Indeed, the numbers are similar enough that each defecation event may flip the ratio to favor human cells over bacteria.

```
Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacte
```

19.0.1.4 Cure/cause obesity by FMT

Several studies in mice hint that an obese microbiome can be transferred to a skinny one and vice versa:

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Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2
31. http://doi.org/10.1038/nature05414
```

19.0.1.5 Moving Pictures

> Here, we present the largest human microbiota time series analysis to date, covering two individuals at four body sites over 396 timepoints.

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Caporaso, J. G., Lauber, C. L., Costello, E. K., Berg-Lyons, D., Gonzalez, A., Stombaugh, J.
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Don't miss the 30-second Youtube summary.

19.0.2 Academic Papers

When you're ready to go to the original sources, be careful: there are tens of thousands of studies, many of them contradictory and quickly out of date. Here are the ones I think deserve to be read first.

- Historic papers (HMG)
- Population studies (enterotype, population studies)
- Specific microbes (Akkermansia, Bifidobacterium, etc.)
- Methods

19.0.3 General Overview

A detailed technical review of how scientists study the microbiome, with an emphasis on how to judge the quality of results. This is a good overview for a smart person who wants an introduction to how we know what we know.

Tyler, Smith, and Silverberg (2014) (Full Text)

Tyler, Andrea D, Michelle I Smith, and Mark S Silverberg. "Analyzing the Human Microbiome: A 'How To' Guide for Physicians." The American Journal of Gastroenterology 109, no. 7 (July 2014): 983–93. doi:10.1038/ajg.2014.73.

Here is another one:

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Young, Vincent B. "The Role of the Microbiome in Human Health and Disease: An Introduction fo
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19.0.4 Microbes and Behavior

A 2019 summary of the links between microbes and psychiatry: Ameringen et al. (2019)

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Ameringen, M., Turna, J., Patterson, B., Pipe, A., Mao, R. Q., Anglin, R., & Surette, M. G.
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19.0.5 Historic Papers

The final paper describing conclusions of the Human Microbiome Project:

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Human, T., Project, M., & Figures, S. (2012). Structure, function and diversity
of the healthy human microbiome. Nature, 486(7402), 207-14. http://doi.org/10.1038/nature1123
```

19.0.6 Self-tracking

Track as much as you can about two people for an entire year: their diet, physical activity, and microbiome; look for correlations. Conclusion: the microbiome is remarkably stable and quickly recovers to its baseline. The "Methods" section is especially interesting because it goes into detail on how to find interesting statistical results with such complicated data.

`David, L. A., Materna, A. C., Friedman, J., Campos-Baptista, M. I., Blackburn, M. C., Perrot`

19.0.7 Diet

Looking for a good overview of studies that link various microbes to diet?

The following two papers are the best summaries:

Scott et al. (2013)

Scott, K. P., Gratz, S. W., Sheridan, P. O., Flint, H. J., & Duncan, S. H. (2013). The influence of diet on the gut microbiota. Pharmacological Research, 69(1), 52–60. http://doi.org/10.1016/j.phrs.2012.10.020

Portune et al. (2017)

Portune, Kevin J., Alfonso Benítez-Páez, Eva Maria Gomez Del Pulgar, Victor Cerrudo, and Yolanda Sanz. "Gut Microbiota, Diet and Obesity-Related Disorders - the Good, the Bad and the Future Challenges." Molecular Nutrition & Food Research, June 2016. doi:10.1002/mnfr.201600252.

Here's another one; see the supplements for details about which foods affect which bacteria.

David, Maurice, et al. (2014)

`David, Lawrence A., Corinne F. Maurice, Rachel N. Carmody, David B. Gootenberg, Julie E. Button, Benjamin E. Wolfe, Alisha V. Ling, et al. "Diet Rapidly and Reproducibly Alters the Human Gut Microbiome." Nature 505, no. 7484 (December 11, 2013): 559-63. doi:10.1038/nature12820.`

19.0.8 Population studies

The American Gut project citizen science survey of more than 10,000 microbiome samples, published its results in 2018, finding very few clear associations between self-reported *anything* (sex, age, diet) and microbial diversity – except one: people who self-reporting eating the most diverse numbers of plants had higher diversity than those who didn't.

McDonald et al. (2018)

McDonald, D., Hyde, E., Debelius, J. W., Morton, J. T., Gonzalez, A., Ackermann, G., … Gunde

19.0.9 Enterotypes

The intriguing idea that there may be identifiable patterns in our microbiomes, called enterotypes, was proposed in this highly-cited paper, which includes a detailed methods supplement to show you how to compute it yourself:

Arumugam, Manimozhiyan, Jeroen Raes, Eric Pelletier, Denis Le Paslier, Takuji Yamada, Daniel
80. doi:10.1038/nature09944.

The idea that identifiable enterotypes may exist has been viewed skeptically in follow-up work.

19.0.10 Large population summaries

Twin studies help tease out the different effects of human and microbial DNA. This is a recent update to a study of 1,126 twin pairs:

Goodrich, Julia K., Emily R. Davenport, Michelle Beaumont, Matthew A. Jackson, Rob Knight, Ca
43. doi:10.1016/j.chom.2016.04.017.

Two excellent papers present a detailed analysis of the microbiomes and associated phenotypic information from several thousand healthy people in the Belgian Flemish Gut Flora Project (N = 1106) and the Dutch LifeLines-DEEP study (N = 1135).

Falony, G., M. Joossens, S. Vieira-Silva, J. Wang, Y. Darzi, K. Faust, A. Kurilshikov, et al. “Population-Level Analysis of Gut Microbiome Variation.” Science 352, no. 6285 (April 29, 2016): 560–64. doi:10.1126/science.aad3503.

Zhernakova, A., A. Kurilshikov, M. J. Bonder, E. F. Tigchelaar, M. Schirmer, T. Vatanen, Z. Mujagic, et al. “Population-Based Metagenomics Analysis Reveals Markers for Gut Microbiome Composition and Diversity.” Science 352, no. 6285 (April 29, 2016): 565–69. doi:10.1126/science.aad3369.

Be sure to study the supplemental materials, especially Supplement Table 11, which includes details of the specific microbes.

19.0.11 Methods

A good overview of the current state of how microbiome analysis is performed, from the sample collection processing, to the data pipeline and final bioinformatics summaries. It includes references to the top platforms (e.g QIME, Mothur, PICRUSt) along with the various tradeoffs of each:

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Amato, Katherine R. "[An Introduction to Microbiome Analysis for Human Biology Applications]
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19.1 Other Resources

Elizabeth Bik keeps an excellent Microbiome Papers Collection of a few dozen classic academic papers.

and you'll find even more in Tyler, Smith, and Silverberg (2014), which is strongly recommended.

19.1.1 Software

ANCOM (Mandal et al. (2015)) is an open source software tool[1] to help understand abundances.

When we compare populations from one ecosystem (e.g. my results on Monday) with another (e.g. my results on Tuesday), there is a fundamental statistical sense in which the two populations are not comparable.

This paper gives the analogy of trying to compare two forests after capturing 100 animals in each: you count 20 bears in one and 30 in the other. There are statistical ways to say with confidence that the first forest is composed of 20% bears and the other 30%, but there is no way to conclude that the second forest has more bears without knowing the total number of animals in each.

A reliance on relative abundances (i.e. percentages) carries other, statistical, problems. For example, the Pearson correlation coefficient is difficult to interpret, since the sum-to-one characteristic of relative abundances requires mathematically that there be some negative correlations. If the numbers were absolute, you wouldn't necessarily have negative correlations.

[1]The R code is here: http://www.niehs.nih.gov/research/resources/software/biostatistics/ancom/index.cfm

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21 Appendix

This is a parking place for content that should be incorporated into other parts of the book

21.1 Healthy Microbiome

2024-10 from Gut Microbiome for Health

> Another new study by Liping Zhao and colleagues at the Rutger Center for Microbiome Analysis, along with international collaborators, has utilized artificial intelligence models for identifying a set of gut microorganisms that play a critical role in digestion, immune responses, and mental health. What is new with the analysis is that it is based on genome-specific analysis and database independence, and it is focused on stable gut microbiome interactions4.
>
> The core microbiome's structure includes two distinct groups of bacteria (i.e., the Foundation Guild and the Pathobiont Guild) that compete with each other as an indicator of health and help differentiate cases from controls across 15 diseases across three continents and predict immunotherapy outcomes. Stable interactions within gut microbiome members appear more relevant than the abundance of microorganisms. These findings open a new potential way to disease prediction and classification and manage microbiome-related diseases through specific interventions that target the core microbiome related to health4.

21.2 Microbes in the air

An international team of researchers found hundreds of different genera of fungi and bacteria at up to 3,000 meters, including many human pathogens: Escherichia coli, Serratia marcescens, Prevotella melaninogenica, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus, Cutibacterium acnes, Clostridium difficile, Clostridium botulinum, Stenotrophomonas maltophilia, Shigella sonnei, Haemophillus parainfluenzae and Acinetobacter baumannii and health-relevant fungi such as Malassezia restricta, Malassezia globosa, Candida parapsilosis and Candida zeylanoides, Sarocladium kiliense, Cladosporium halotolerans, and Cladosporium herbarum.

https://www.pnas.org/doi/10.1073/pnas.2404191121

21.3 Diet Effectiveness Depends on Microbes

Stanford's Michael Snyder and his lab found Distinct factors associated with short-term and long-term weight loss induced by low-fat or low-carbohydrate diet intervention

> Interestingly, we observe minimal dietary differences between those who succeeded in long-term weight loss and those who did not. Instead, proteomic and gut microbiota signatures significantly differ between these two groups at baseline.

21.4 Database of Food Microbes

Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome Carlino, NiccolòAlvarez-Ordóñez, Avelino et al. Cell, Volume 0, Issue 0

From *Cell* August 2024:

> Here, we present curatedFoodMetagenomicData (cFMD), an open-access resource that collects food-associated microbial data to support the use of metagenomics in food science. The current release comprises 2,533 food metagenomes with standardized metadata, 1,950 of them newly sequenced within the MASTER EU Consortium. We generated 10,112 prokaryotic and 787 eukaryotic metagenome-assembled genomes (MAGs) from food that were grouped into 1,036 prokaryotic and 108 eukaryotic species clusters, 320 of which resulted to be uncharacterized when compared with >1 M existing genomes. We included these MAGs into our pipelines for sensitive taxonomic profiling and applied it to 19,833 human metagenomes, revealing species- and strain-level overlaps along the food-human axis.

21.5 Microbes in Your Bathroom

Northwestern University scientists poured through the microbes in a typical bathroom showerhead and toothbrush to find these places harbor numerous unidentified organisms, with surprisingly little overlap among the different environments.

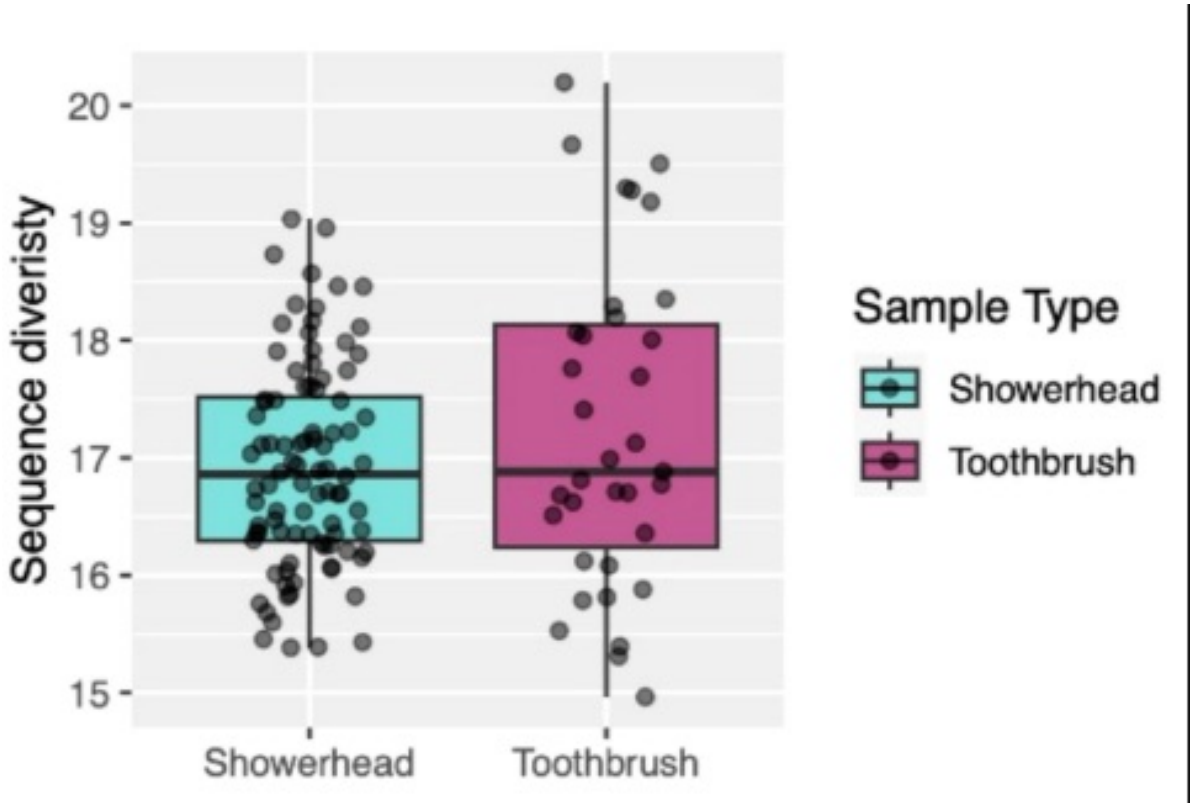

Figure 21.1: A toothbrush has more diversity than a showerhead

Front. Microbiomes, 08 October 2024 Sec. Environmental Microbiomes Volume 3 - 2024 | https://doi.org/10.3389/frmbi.2024.1396560

Phage communities in household-related biofilms correlate with bacterial hosts

21.6 Microbes in Your Microwave Oven

Your microwave oven has its own microbiome

> Alba Iglesias, a microbiologist at the University of Valencia in Spain, and her colleagues swabbed 30 microwave ovens A total of 101 bacterial strains grew in the cultures. The dominant ones belonged to the Bacillus, Micrococcus and Staphylococcus genera, which commonly live on human skin and surfaces that people frequently touch. Human-skin bacteria were present in all three types of microwave oven, but were more abundant in the household and shared-use appliances. A few bacteria types associated with food-borne illnesses, including Klebsiella and Brevundimonas, also grew in some of the cultures from household microwaves.

Iglesias, A., Martínez, L., Torrent, D. & Porcar, M. Front. Microbiol. https://www.frontiersin.org/journals/micr (2024).

21.7 Microbes Help Animals Detect Magnetic Fields

University of Central Florida biologist Robert Fitak created a refined database of magnetic bacteria speculating that it's microbes that help turtles and other animals navigate.

Animals' Magnetic 'Sixth' Sense May Come from Bacteria, New Paper Suggests

> Fitak found, for the first time, that magnetotactic bacteria are associated with many animals, including a penguin species, loggerhead sea turtles, bats and Atlantic right whales. For instance, Candidatus Magnetobacterium bavaricum regularly occurred in penguins and loggerhead sea turtles, while Magnetospirillum and Magnetococcus regularly occurred in the mammal species brown bats and Atlantic right whales.

They speculate that the microbes might live somewhere in the nervous tissue of these animals.

21.8 Most Microbes are Dormant

An estimated 60% of all microbes are lying dormant at any given moment, ready to be switched back to life when conditions are right.[1]

A natural protein, Balon, latches onto ribosomes to lock it in place and shut down the cell. Balon is found in around 20% of all microbes, and even more microbes might harbor genes that behave similarly.

21.9 Microbes Spread Through Dust Storms

In Unseen travelers: Dust storms may spread bacteria and fungi around the world

> Dr. Shankar Chellam, professor in the Zachry Department of Civil and Environmental Engineering and A.P. and Florence Wiley Professor III at Texas A&M University, and his now former student Dr. Sourav Das have furthered previous research to identify microorganisms that might have hitched a ride in the dust

[1] see 'Most Life on Earth Is Dormant, After Pulling an 'Emergency Brake'' in https://www.quantamagazine.org/most-life-on-earth-is-dormant-after-pulling-an-emergency-brake-20240605

plumes across the Atlantic. Dr. Daniel Spalink, assistant professor and director of S.M. Tracy Herbarium at Texas A&M, helped analyze the biology and identify bacteria and fungi in the samples.

21.10 Human Milk as a Treatment for Gut Disease

Human milk oligosaccharides (HMOs), the third most abundant component of human milk are not found in other kinds of milk, yet play a vital role in the care and feeding of *Bifidobacterium*.

Prolacta Bioscience is one company that makes a human-equivalent HMO intended to feed gut bacteria, and perhaps, solve numerous gut problems associated with dysfunctional microbiomes.

Seattle-based Intrinsic Medicine is currently running on clinical trial to use HMO-based drugs for treatment of Parkinsons Disease.

From Stat News Mar 2024

> Biomilq, for instance, synthesizes milk using mammary cells for people who have difficulty breastfeeding. And adults have occasionally tried to tap those benefits via dietary or nutritional supplements — and even by obtaining colostrum, the first milk produced by mammals within the first 48 to 72 hours after birth. Companies such as Armra are marketing colostrum, albeit from a bovine source, in powder form.

Longitudinal profiling of the microbiome at four body sites reveals core stability and individualized dynamics during health and disease

Mentions uBiome's Melissa Agnello for processing some samples through uBiome's lab.

Longitudinal profiling of the microbiome at four body sites reveals core stability and individualized dynamics during health and disease

Xin Zhou, Xiaotao Shen, Jethro S. Johnson, Daniel J. Spakowicz, Melissa Agnello, Wenyu Zhou, Monica Avina, Alexander Honkala, Faye Chleilat, Shirley Jingyi Chen, Kexin Cha, Shana Leopold, Chenchen Zhu, Lei Chen, Lin Lyu, Daniel Hornburg, Si Wu, Xinyue Zhang, Chao Jiang, Liuyiqi Jiang, Lihua Jiang, Ruiqi Jian, Andrew W. Brooks, Meng Wang, Kévin Contrepois, Peng Gao, Sophia Miryam Schüssler-Fiorenza Rose, Thi Dong Binh Tran, Hoan Nguyen, Alessandra Celli, Bo-Young Hong, Eddy J. Bautista, Yair Dorsett, Paula Kavathas, Yanjiao Zhou, Erica Sodergren, George M. Weinstock, Michael P. Snyder bioRxiv 2024.02.01.577565; doi: https://doi.org/10.1101/2024.02.01.57756

21.11 Behavior

21.11.1 Social Transmission of Microbiomes

A 2024 study by Yale Network Scientist Nicholas Christakis says Your friends shape your microbiome — and so do their friends. They mapped the social networks and microbiomes of almost 2000 people in rural Honduras villages and found—sure enough—that people who interact regularly have more similar microbes than those who don't.

Full text: Beghini, F., Pullman, J., Alexander, M. et al. Gut microbiome strain-sharing within isolated village social networks. Nature (2024). https://doi.org/10.1038/s41586-024-08222-1

21.11.2 Alcholism and Gut Microbes

Could the gut give rise to alcohol addiction? asks *Nature*, describing work on Alcohol Use Disorder by Sophie Leclercq, a biomedical scientist at the Catholic University of Louvain in Brussel, and others.

> Leclercq thinks that 30–40% of cases of AUD might have a gut-related component that could be targeted for treatment. A key challenge is determining exactly which components to target — it is as yet unclear what constitutes a 'good' microbiome. Day's analysis suggests that bacteria such as Lactobacillus, were in abundance in people with AUD, whereas Akkermansia and some others were low.
>
> The gut bacteria Lactobacillus, for example, can produce GABA; Enterococcus can produce serotonin; and Bacillus can make dopamine. Short-chain fatty acids (SCFAs) released when dietary fibre is fermented by bacteria in the gut also have neuroactive properties.

Review article (2023): Gut Microbiota in Anxiety and Depression: Unveiling the Relationships and Management Options

> A greater incidence of depression is substantially linked to a lower protein consumption than recommended. A 10% increase in protein consumption was shown to reduce the incidence of depression considerably in South Korea and in the United States. Several biological explanations have linked the intake of protein and depression. These theories are supported by the fact that tryptophan, an amino acid, is a precursor of serotonin.

21.12 Beyond Microbes

'Obelisks': Entirely New Class of Life Has Been Found in The Human Digestive System Stanford University biologist Ivan Zheludev searched millions of published genome and identified at least 30K different Obelisks that appeared in about 10% of the samples.

It's RNA with only 1000 nucleotides.

Viroid-like colonists of human microbiomes Ivan N. Zheludev, Robert C. Edgar, Maria Jose Lopez-Galiano, Marcos de la Peña, Artem Babaian, Ami S. Bhatt, Andrew Z. Fire bioRxiv 2024.01.20.576352; doi: https://doi.org/10.1101/2024.01.20.576352

21.13 Diversity and Immunity

21.14 Statistically Modeling for Health Status

Zhu, J., Xie, H., Yang, Z. et al. Statistical modeling of gut microbiota for personalized health status monitoring. Microbiome 11, 184 (2023). https://doi.org/10.1186/s40168-023-01614-x

Statistical modeling of gut microbiota for personalized health status monitoring

> We systematically developed a statistical monitoring diagram for personalized health status prediction and analysis. Our framework comprises three elements: (1) a statistical monitoring model was established, the health index was constructed, and the health boundary was defined; (2) healthy patterns were identified among healthy people and analyzed using contrast learning; (3) the contribution of each bacterium to the health index of the diseased population was analyzed. Furthermore, we investigated disease proximity using the contribution spectrum and discovered multiple multi-disease-related targets.

via Ken Lassessen

21.15 Eye Disease and Gut Microbiome

Some inherited eye diseases, including blindness may be caused by gut bacteria that

> RB1 gene is key to controlling the integrity of the lower gastrointestinal tract, the first ever such observation. There, it combats pathogens and harmful bacteria by regulating what passes between the contents of the gut and the rest of the body.
>
> The team found that when the gene has a particular mutation, dampening its expression (reducing its effect), these barriers in both the retina and the gut can

> be breached, enabling bacteria in the gut to move through the body and into the eye, leading to lesions in the retina that cause sight loss.

The research was conducted on mice by Professor Richard Lee (UCL Institute of Ophthalmology and Moorfields Eye Hospital NHS Foundation Trust).

https://www.cell.com/cell/abstract/S0092-8674(24)00108-9

21.16 Skin Microbiome and Attractiveness to Mosquitoes

Skin microbiome alters attractiveness to Anopheles mosquitoes

> Staphylococcus 2 ASVs are four times as abundant in the highly-attractive compared to poorly-attractive group.

via Axios

21.17 Ticks and Alpha-Gal

see Ticks, Alpha-Gal, Neu5gc and more

The CDC warns about

> Alpha-gal syndrome (AGS) is a serious, potentially life-threatening allergic condition. AGS is also called alpha-gal allergy, red meat allergy, or tick bite meat allergy.

does it have an association with microbes?

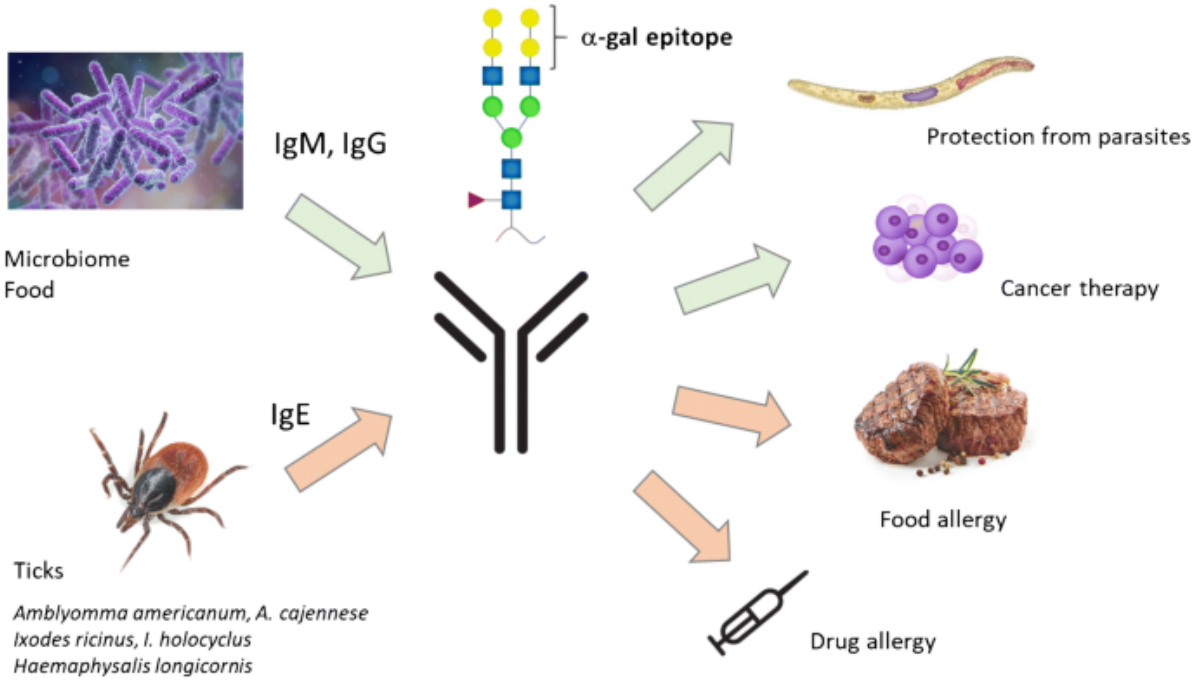

Figure 21.2: Figure 2: IgM and IgG antibodies are generated by continuous stimulation by the intestinal microbiome and probably also by food

21.18 Gut-Brain

Science Magazine

> a new study reveals the gut has a much more direct connection to the brain through a neural circuit that allows it to transmit signals in mere seconds.

21.19 Paleo Humans

Natural products from reconstructed bacterial genomes of the Middle and Upper Paleolithic from a German team that > Now, a new study from an interdisciplinary team has taken important steps to understanding stone age bacteria by sequencing genomes recovered from ancient dental calculus. The hardened tartar preserved bacterial fragments on the teeth of 12 Neanderthals and 34 humans that had lived anywhere from 102,000 to 150 years ago. Formed from plaque, this calculus fossilized during these humans' lifetime, trapping genetic fragments inside.

Discover Magazine on Bacterial DNA from ancient humans

21.20 Microbiome uniqueness

See https://pubmed.ncbi.nlm.nih.gov/30150716/

A Chinese study that found microbiome patterns that predict health in one province don't work in another.

He et al. (2018)

He Y, Wu W, Zheng HM, Li P, McDonald D, Sheng HF, Chen MX, Chen ZH, Ji GY, Zheng ZD, Mujagond P, Chen XJ, Rong ZH, Chen P, Lyu LY, Wang X, Wu CB, Yu N, Xu YJ, Yin J, Raes J, Knight R, Ma WJ, Zhou HW. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nat Med. 2018 Oct;24(10):1532-1535. doi: 10.1038/s41591-018-0164-x. Epub 2018 Aug 27. Erratum in: Nat Med. 2018 Sep 24;: PMID: 30150716.

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https://www.nature.com/articles/s42255-021-00348-0

see evernote

Wilmanski et al. (2021)

21.21 Methods

Greengenes2 unifies microbial data in a single reference tree

> Studies using 16S rRNA and shotgun metagenomics typically yield different results, usually attributed to PCR amplification biases. We introduce Greengenes2, a reference tree that unifies genomic and 16S rRNA databases in a consistent, integrated resource. By inserting sequences into a whole-genome phylogeny, we show that 16S rRNA and shotgun metagenomic data generated from the same samples agree in principal coordinates space, taxonomy and phenotype effect size when analyzed with the same tree.

21.22 Mapping the Capacity of a Single Subject's Microbiome to Metabolize Hundreds of Drugs

57 drugs that are transformed by the microbiome, with lots of variance from person to person.

https://www.cell.com/cell/fulltext/S0092-8674(20)30563-8#secsectitle0035

Javdan et al. (2020)