

bm211-workshops

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Preface to the 2023-24 presentation

Welcome to the 2023-24 edition of the BM211 computing workshops.

This is the first presentation of this material in this form, and we would be very grateful to hear feedback [by email](#) or through the [GitHub repository Issues page](#).

1 Introduction

This is a book created from markdown and executable code.

See Knuth (1984) for additional discussion of literate programming.

```
1 + 1
```

```
[1] 2
```

2 Summary

In summary, this book has no content whatsoever.

1 + 1

[1] 2

Part I

Workshop 01

Our goal in this computational workshop is to introduce some concepts in microbial ecology.

3 Microbial Ecology

This section introduces key concepts of how we use molecular techniques to identify and quantify the composition of microbial communities, to study microbial ecology.

Note

This is accompanying material to give additional background and explanation for the workshop. You don't *need* to read this for the workshop itself as we will be covering the material in person, but we hope you find it helpful.

3.1 What is microbial ecology?

Microbial ecology is the scientific study of natural microbial communities. It is a hugely important, but arguably underdeveloped area of biology. Important questions remain to be completely answered in detail for many biological systems:

The composition of microbial communities

- what microbes are found in a community?
- what proportion of each microbe species (or other group) is present in a community?
- what are the physical limits of the community?

interactions within the community

- is the community *stable* (or is it growing, shrinking, or changing composition)?
- do community members share or compete for resources, and which resources?
- do the community members effectively operate as a larger-scale system?

interactions of a community with other organisms

- does the gut microbial community influence the host animal's nutrition, health, or disease status?
- does the rhizosphere microbial community influence a plant's ability to extract

nutrition or energy from the environment, and contribute to protection against pathogens?

💡 Interactions between communities

- when two communities come together, what does the resulting community look like?
- are there two or more separate (or interacting communities), or do they somehow combine?

💡 Engineering new microbial communities

- can we predict which microbes can combine into a productive community?
- can we choose these microbes to engineer a community that achieves a particular goal (e.g. plant protection or gut health)
- can we perturb existing communities to make them beneficial to health or achieve some other goal?

This array of questions can be summarised more simply as (Prosser 2020):

1. Who is there?
2. What is meant by “there”?
3. Who is doing what in there?
4. What is the effect of doing ... to “there”?

3.2 Answering questions about microbial ecology using molecular techniques

Molecular techniques enabled by modern sequencing approaches, such as amplicon marker sequencing (e.g. 16S, ITS1 methods) - also known as **metabarcoding** - and sequencing the entire genomic DNA of a complex sample - also known as **metagenomics** - have become the dominant approach to understanding microbial community composition (Boughner and Singh 2016). These tools give rapid insights into the complex composition of microbial communities that are unattainable using laborious, time-consuming culture-based techniques.

🔥 Metagenomics *vs* Metabarcoding

The two words are quite similar, but it is important to distinguish between

- **metabarcoding:** sequencing a single marker sequence from a population
- **metagenomics:** deep sequencing of the entire genomic DNA material in a sample,

often attempting to assemble representative genomes from the data

In addition to their speed and the scale of data they can produce, these molecular techniques have other advantages:

- The (sequence) data collected can be preserved and shared, alongside metadata describing the experiment, in public databases. This enables reanalysis and integration into larger-scale studies.
- Fastidious organisms, or those that might be outcompeted in a laboratory culture, can be sequenced and identified.
- Organisms present in low numbers can be sequenced and identified.

! Important

These molecular techniques answer the question of “*Who is there?*”, in two ways:

1. They tell us **what kinds of organism are present** (often to *species* or *genus* level)
2. They give us a relative count of **how much of each kind of organism is present**

Taken together, these answers tell us the **diversity of the community**.

i Note

We will introduce measures of community diversity in [a later section](#).

3.2.1 Which organisms are present?

Both **metabarcoding** and **metagenomics** can tell us which microorganisms are present in a biological or environmental sample.

3.2.1.1 Metabarcoding

In **metabarcoding**, we use the polymerase chain reaction (PCR) and **primers targeted specifically to amplify a marker sequence**: a gene fragment or other stretch of DNA that we believe to be present in all organisms of interest. For bacteria, this is usually a fragment of the 16S rRNA gene. For fungi and other eukaryotic microorganisms it may be a fragment of the ITS1 (Internally Transcribed Spacer 1) region.

These regions of the genome acquire sequence changes as their organisms evolve over time, such that variants of the marker sequence can be associated with a particular group of microorganisms, usually at the species or genus level. By amplifying and then sequencing the marker sequence from all suitable organisms in the sample, we can **identify the marker sequence variants, and then compare these to databases of known sequences**, to identify which organisms are present.

3.2.1.2 Metagenomics

A similar principle applies with **metagenomics**: we sequence the sample and then compare the resulting sequences to a database containing sequences of known organisms, and use this to identify which of them are present in our sample. Differences between the methods arise mainly because **this approach is untargeted - we do not know in advance what sequences we will recover** - and generates very large amounts of data. Three of the main consequences of this are:

1. We can compare individual sequencing reads to the database of known sequences, but due to the large amount of sequence data (in comparison to metabarcoding) we have to do this using special techniques like **k-mer alignment**, rather than direct sequence comparison (Wood and Salzberg 2014).
2. We can assemble near-complete and complete genomes from the data: **Metagenome-Assembled Genomes (MAGs)**. (Setubal 2021).
3. We can obtain much more information than simple organism identity, such as the presence or absence of individual genes or gene functions, such as antimicrobial resistance genes (Abreu, Perdigão, and Almeida 2020).

3.2.2 How much of each organism is present?

Whichever method we use to sequence a biological sample, we can obtain a list of the organisms that are present. This tells us something about the composition of the sample, but not everything. There is clearly a difference between one community that is 50% *Escherichia coli* and 50% *Staphylococcus aureus*, and another community that is 99% *S. aureus* and 1% *E. coli*. But how can we distinguish between the two?

In both metabarcoding and metagenomics we can count the number of sequences that correspond to each of the organism types we have identified. For metabarcoding, this might be the count of each amplicon sequence corresponding to a microbial genus. For metagenomics, this might be the number of reads assigned to a microbial species by a tool like [Kraken](#).

 Molecular techniques only *estimate* organism abundance

All counts of organisms, by both methods, are strictly **estimates of the representation of each organism in the sample**. There are many factors that influence the way this estimate may vary from the actual amount or proportion of any organism in the sample, including:

- DNA quality (Manzari et al. 2020)
- laboratory technique (sample spillover or other handling problems)
- PCR artefacts (especially for low-biomass samples)
- sequence database integrity
- variable accuracy of associating a sequence with an organism or group (taxonomic misassignment)

With metagenomic analyses that result in assembled MAGs, an additional way of estimating the amount of each organism present is possible: we can count the number of reads that contribute to each MAG assembly. These reads are then assigned the same identity as that of the MAG itself.

We refer to the count of each distinct group of microbes as its **abundance**.

3.3 Describing community composition

The list of organisms identified as present in our sample and the (relative) count of how much of the community is composed of each organism, taken together, describe our measured **microbial community composition**.

4 Diversity Measures

Note

This section introduces the concept of a **diversity measure** in microbial ecology.

- We introduce an **example community**, obtained from Mars, to explore concepts with.
- We discuss fundamental concepts in ecological diversity:
 - **richness**
 - **evenness**

4.1 What is diversity?

Diversity measurements for a microbial community are quantitative estimates of how *heterogeneous* or *homogeneous* that community is. Qualitative estimates of diversity can be simple. If a community is composed of identical individuals, it is clearly not diverse. However, if the members of a community differ from each other, the community is diverse to some extent.

Important

Diversity (or the lack of it) is a **property of the community, not a property of individuals** in the community.

If we want to record the level of diversity in a community, or compare it to other communities, we cannot easily rely on qualitative estimates. We need to be more precise than this, and to use *quantitative* approaches. We need to know what groups are present in the community and, numerically, to what extent each group is represented.

4.1.1 An example community

To help illustrate this, we went to Mars¹ to obtain a sample (Figure 4.1).



Figure 4.1: A community sample, obtained from Mars

This community contains a range of individuals of different type, distinguishable by the detailed structure of their outer membranes, and also by their internal contents. The diversity of the community can be seen in (Figure 4.2).

Exercise 1

By visual inspection of Figure 4.2, how diverse would you say the sample is?

Answer

There are clearly several different kinds of individual present, so the sample is not homogenous and is *diverse* in some sense. But in another sense the individuals are all individually-wrapped sweets, so the sample is entirely homogeneous.

We clearly need to be more precise about what exactly we mean, when talking about *diversity*, so that others can understand exactly what we mean.

¹OK, we actually went to Sainsbury's Local. The one on Buchanan Street. But the sweets *are* technically from *Mars*.

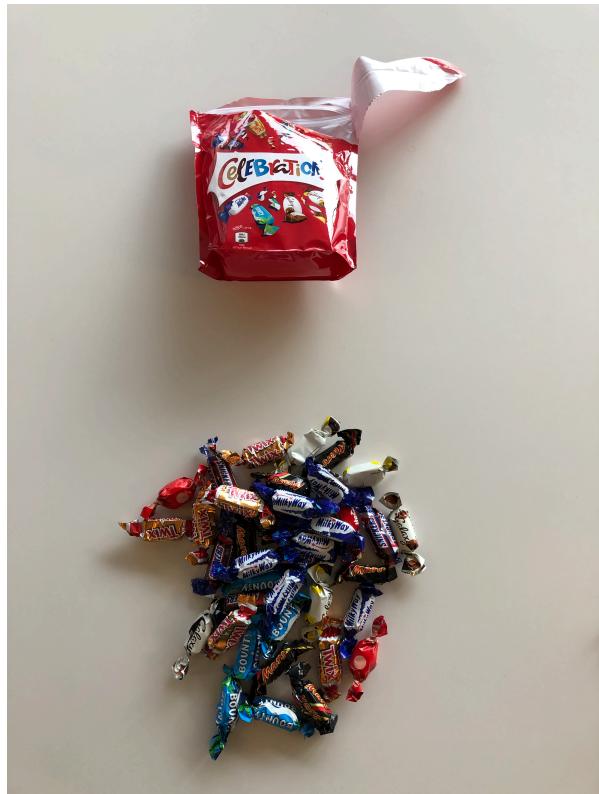


Figure 4.2: A detailed view of the Mars sample, showing community diversity

4.2 Community richness and evenness

We usually define how *diverse* a community is by using a **diversity index**. This is a number that reflects the distribution of different *types* (such as microbial species) of individual that are found in a sample. There are two major concepts that contribute to diversity indices.

💡 Richness

The **richness** of a sample is the **number of different types that are represented in the sample**.

For example, *species richness* is a count of the total number of species in the sample. It **does not take into account species abundance, or relative abundance**.

💡 Evenness

The **evenness** of a sample reflects **how similar is the abundance of each type of thing** in the sample. The more similar the proportion of each type, the more *even* the community.

For example, *species evenness* reflects the relative abundances of distinct species in the sample. If a sample contains three species: *A*, *B*, and *C*, then:

- a sample with 33% *A*, 33% *B*, and 33% *C* has **high species evenness**
- a sample with 5% *A*, 90% *B*, and 5% *C* has **low species evenness**

4.3 Richness and evenness in the Mars sample

We can organise the individuals in the Mars sample, as shown in Figure 4.3. This gives us enough information to calculate species richness and evenness for this community.

4.3.1 Species richness

We can easily count the number of different species, as determined by morphology and membrane features, in Figure 4.3. There are eight (8) species, and this is a quantitative measure of species richness.

Taken in isolation though, this number doesn't tell us very much. We would need other information - maybe from other samples - to know whether eight is a large, small, or intermediate number of species. Nor does this number tell us whether all species are represented to the same extent (even if, from looking at Figure 4.3 we can tell they are not). We need to consider *evenness* for that.

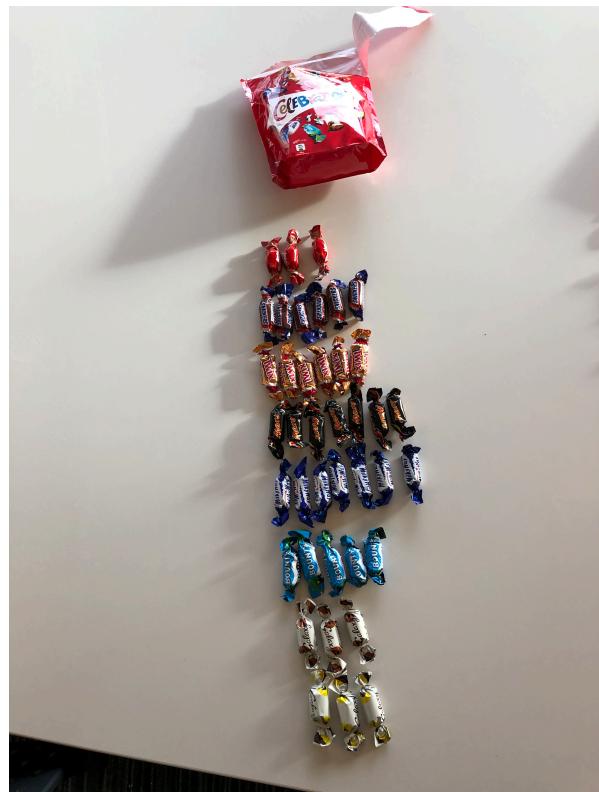


Figure 4.3: Species abundance in the Mars sample community

4.3.2 Species evenness

We can see visually from Figure 4.3 that there are not equal amounts of each species, so the community is not perfectly even in terms of species evenness. **But the question remains open:** how do we combine richness and evenness together into an *index* or number, to understand species diversity in our sample?

! Important

We will discuss a simple measure of species diversity, *Shannon Index*, in [the next chapter](#).

5 Shannon Index

Note

This section introduces the **Shannon Index** as a simple measure of community diversity.

- We introduce the concept of **Shannon Index** and explain what it means.
- **We walk through calculation of the Shannon Index in R**, using data from our example community in Chapter 4.
 - We relate the actions in our R walkthrough to the equation for Shannon Index, at each step.
- We introduce the concept of **Effective number of species** in a community and how this relates to Shannon Index.

To understand diversity, we need to consider both richness and evenness, as described in Chapter 4.

We can obtain a simple count of species to calculate richness. But we also know that if each species is present in about the same abundance there is *high evenness*, and if the population is dominated by a single species there is *low evenness*. But by itself this *idea of evenness does not give us a number that we can use to compare communities*.

There are mathematical formulae that allow us to turn this concept of evenness into a number, and we can calculate such values for a community using R.

5.1 What is the Shannon index?

The **Shannon Index (H)** is arguably the simplest quantitative measure of community evenness/diversity, and is described by Equation 5.1. Shannon Index varies between:

- a value close to zero (for very uneven communities)
- the value $\ln(N)$ for a community with N evenly-distributed species.

The way that the maximum value of Shannon Index varies with species richness (count of species) is shown in Figure 5.1.

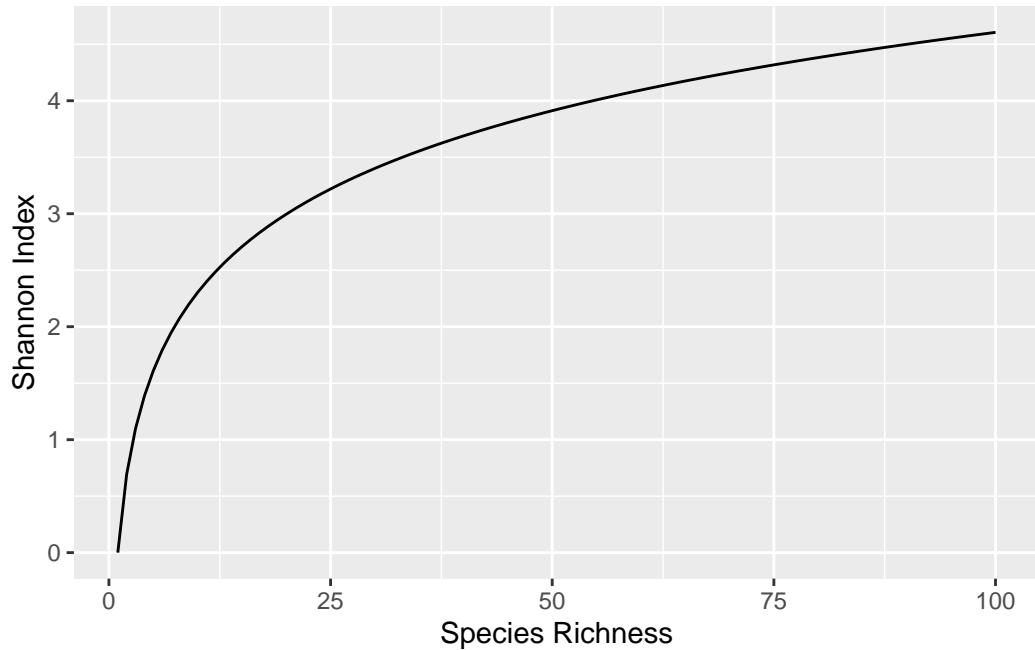


Figure 5.1: Plot of maximum Shannon Index against community richness. A value close to zero for Shannon Index always indicates that the community is highly uneven and not very diverse. The closer the value of Shannon Index gets to the line, for a community of given richness, the closer that community is to being maximally diverse (defined as an even distribution of species).

$$H = - \sum_{i=1}^N p_i \ln(p_i) \quad (5.1)$$

5.1.1 What does the equation for Shannon Index mean?

The Shannon Index describes the *evenness* of species in a community. Representing a single species from the community as the letter i , we can calculate the **relative abundance** of that species in the community as its **abundance** (the count of individuals from species i) divided by the total abundance for all species. We call this relative abundance p_i .

Equation 5.1 takes this value and transforms it into the Shannon Index, represented as H . The resulting value varies between zero (maximally uneven, not diverse), and some maximum value (maximally even, very diverse), as represented in Figure 5.1. Knowing the *richness* (total number of species) for a community and the *Shannon Index* (H), we can quantify how diverse our community is.

5.2 Calculating Shannon Index for our Mars Community

5.2.1 Collecting data about our community

Let's start in R by defining our dataset for the Mars community.

In the code below we create two vectors: one of species names, and one of counts for those species in our sample (which we can get from Figure 4.3). We combine these vectors into a single dataframe, for convenience.

```
# Define a vector of species names
species <- c("Malteaser sp.", "Snickers sp.", "Twix sp.", "Mars sp.",
           "Milky way", "Bounty sp.", "Galaxy choc", "Galaxy caramel")

# Define a vector of corresponding species counts
count <- c(3, 6, 6, 7, 7, 5, 3, 3)

# Bring these together in a dataframe
community.df <- data.frame(species, count)
```

| species | count |
|-----------|-------|
| Malteaser | 3 |
| sp. | 3 |

| species | count |
|-------------------|-------|
| Snickers sp. | 6 |
| Twix sp. | 6 |
| Mars sp. | 7 |
| Milky way | 7 |
| Bounty sp. | 5 |
| Galaxy choc | 3 |
| Galaxy caramel | 3 |

5.2.2 Calculating relative abundance

The first step in calculating Shannon Index is to calculate the **relative abundance** p_i of each species i . To do this, we calculate the percentage of the entire community that is made up from each species, as below. We divide the count for each species by the sum of counts for all species (i.e. the total number of individuals, in this case).

```
# Calculate relative abundances
community.df$rel_abd <- community.df$count / sum(community.df$count)
```

| species | count | rel_abd |
|------------------|-------|---------|
| Malteaser sp. | 3 | 0.075 |
| Snickers sp. | 6 | 0.150 |
| Twix sp. | 6 | 0.150 |
| Mars sp. | 7 | 0.175 |
| Milky way | 7 | 0.175 |
| Bounty sp. | 5 | 0.125 |
| Galaxy choc | 3 | 0.075 |

| species | count | rel_abd |
|----------------|-------|---------|
| Galaxy caramel | 3 | 0.075 |

🔥 Mathematical Content!

How does what we've just done in R relate to Equation 5.1?

For each of our species (denoted by i in the equation), we have calculated the relative abundance p_i , which is used in the part of Equation 5.2 indicated in red, below.

$$H = - \sum_{i=1}^N \textcolor{red}{p_i} \ln(p_i) \quad (5.2)$$

5.2.3 Calculating Shannon Index

To turn this data into the Shannon Index, we need to carry out two more steps: calculate the *natural log* of the relative abundance of each species ($\ln(p_i)$), then multiply this by the corresponding relative abundance (p_i), as in the R code below:

```
# Calculate the natural log of relative abundance
community.df$ln_rel_abd <- log(community.df$rel_abd)

# Multiply the relative abundance by its natural log
community.df$mult <- community.df$rel_abd * log(community.df$rel_abd)
```

| species | count | rel_abd | ln_rel_abd | mult |
|---------------|-------|---------|------------|------------|
| Malteaser sp. | 3 | 0.075 | -2.590267 | -0.1942700 |
| Snickers sp. | 6 | 0.150 | -1.897120 | -0.2845680 |
| Twix sp. | 6 | 0.150 | -1.897120 | -0.2845680 |
| Mars sp. | 7 | 0.175 | -1.742969 | -0.3050196 |
| Milky way | 7 | 0.175 | -1.742969 | -0.3050196 |
| Bounty sp. | 5 | 0.125 | -2.079442 | -0.2599302 |

| species | count | rel_abd | ln_rel_abd | mult |
|----------------|-------|---------|------------|------------|
| Galaxy choc | 3 | 0.075 | -2.590267 | -0.1942700 |
| Galaxy caramel | 3 | 0.075 | -2.590267 | -0.1942700 |

🔥 Mathematical Content!

Here's how these two actions relate to Equation 5.1.

Firstly, for each of our species, we calculated the natural log of the relative abundance: $\ln(p_i)$, shown in red in Equation 5.3.

$$H = - \sum_{i=1}^N p_i \ln(p_i) \quad (5.3)$$

Next, we calculated the product of p_i and $\ln(p_i)$, highlighted in orange in Equation 5.4.

$$H = - \sum_{i=1}^N p_i \ln(p_i) \quad (5.4)$$

The Shannon Index is then the sum of this final column of values (multiplied by -1 to make it a positive value).

```
shannon_index = -sum(community.df$mult)
shannon_index
```

[1] 2.021916

🔥 Mathematical Content!

Summing the product $p_i \ln(p_i)$ for each species i , and multiplying it by -1 is indicated by the part of Equation 5.5 highlighted in red.

$$H = - \sum_{i=1}^N p_i \ln(p_i) \quad (5.5)$$

5.2.4 Understanding the Shannon Index

So, we have a number $H = 2.02$ as the Shannon Index for our sample. How do we interpret this?

Remember from earlier that the maximum Shannon Index - corresponding to maximum diversity - for a sample with eight species is $\ln(8) = 2.08$. **The value we calculated here, $H = 2.02$, is close to this value and so we can conclude that our sample is very diverse.**

! Important

There is another way to think about this number, called the **effective number of species**.

5.3 The Effective Number of Species for a community

We can use the Shannon Index to calculate a value known as the **effective number of species** for a community. This name reflects that, although a community might contain a certain number of species, **if some species are only present in very low abundance, they are not contributing significantly to the community, and they are effectively not present**, and the effective number of species is less - maybe much less - than the *richness* of the community.

For example:

- A community with three species in equal proportions, e.g. 33% *A*, 33% *B*, and 33% *C* clearly has three equally-contributing species. We might expect the effective number of species to be three.
- A community dominated by a single species, e.g. 1% *A*, 1% *B*, and 98% *C* might *effectively* have only one species: species *C*.

By taking the *exponential* of the Shannon Index, we calculate a value known as the **effective number of species**, or **Diversity (D)** of a community. The mathematical equation is given in Equation 5.6.

$$D = \exp(H) \quad (5.6)$$

🔥 Mathematical content

This works because, as we noted earlier, a community with N evenly-represented species has a Shannon Index of $H = \ln(N)$. Hence, when this is true:

$$D = \exp(H) = \exp(\ln(N)) = N$$

and so, in this special case of absolute evenness, **effective number of species is the same as the actual number of species.**

5.3.1 The Effective Number of Species for our Mars sample

The Shannon Index for our Mars sample was 2.021916, and we can use R to calculate a corresponding Diversity value D by taking the exponential of this number:

```
diversity = exp(2.021916)
diversity
```

```
[1] 7.552782
```

This gives a value of $D = 7.55$ for the effective number of species. This is close to the actual number of species in our sample - the **richness** value - of eight, which indicates that **our sample is highly diverse.**

5.4 Exercises

Exercise 1

Suppose we have a microbial community with the following composition, and counts:

- *Abiotrophia*: 1.27e4
- *Peptostreptococcus*: 1.84e3
- *Corynebacterium*: 2.41e5
- *Eubacterium*: 4.92e3
- *Moraxella*: 7.31e4
- *Neisseria*: 1.88e6
- *Campylobacter*: 8.23e4
- *Desulfovibrio*: 3.11e6

! Important

Use the interactive R box below to calculate the Shannon Index and the Effective Number of Species in the community.

Do you think the community is diverse?

```
# Create a dataframe describing the community
```

```
# Calculate the Shannon Index and Effective Number of Species
```

Answer

The Shannon Index for this community is $H = 0.97$, and the Effective Number of Species is $D = 2.64$.

As we know there are eight species (technically-speaking, taxonomic *genera*) in the community, and the effective number of species is much lower than this, we would conclude that the community is dominated by a small number of genera.

💡 Example solution code

```
# Create a dataframe describing the community
dfm <- data.frame(species=c("Abiotrophia", "Peptostreptococcus",
                           "Corynebacterium", "Eubacterium", "Moraxella",
                           "Neisseria", "Campylobacter", "Desulfovibrio"),
                     count=c(1.27e4, 1.84e3, 2.41e5, 4.92e3, 7.31e4, 1.88e6,
                           8.23e4, 3.11e6))
dfm$p.i <- dfm$count / sum(dfm$count)
dfm$h.i <- dfm$p.i * log(dfm$p.i)

# Calculate the Shannon Index and Effective Number of Species
H = -sum(dfm$h.i)
D = exp(H)

c(H, D)
```

Part II

Workshop 04

Our goal in this workshop is to introduce you to handling and analysing CRISPR-Cas sequence data.

PILER-CR

This chapter will introduce the software tool **PILER-CR**, which is designed to predict the location and content of CRISPR repeats in microbial genomes.

Part III

Workshop 05

Our goal in this workshop is to introduce you to producing and interpreting phylogenetic trees.

Protein Trees

This chapter will guide you through the process of producing a phylogenetic tree from a protein sequence alignment.

References

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