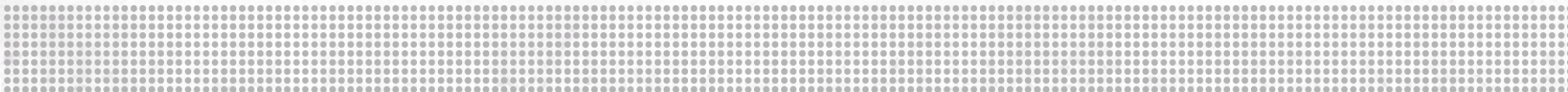


Concepts around microbiome data and statistical considerations



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Learning objectives

- Understand that answering your research question likely requires using various analytic and statistical methods
- Be able to describe typical characteristics of count data (what are rows vs columns, sparse, compositional, etc.)
- Understand typical considerations for cleaning up count data



Outline

- What is a count table?
- What is unique about a sequencing count data?
 - Sparse
 - Multidimensional
 - Compositional
- What are some techniques to overcome these challenges?
 - Transformation
 - Dimension reduction
 - Model selection



The "count table"

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1		Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	taxonomy															
2	ASV1	405	178	545	357	143	829	698	881	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio															
3	ASV2	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
4	ASV3	634	111	940	352	179	545	887	92	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; NA; NA															
5	ASV4	837	733	858	905	127	312	655	796	Bacteria; Firmicutes; Clostridia; Clostridiales; unassigned Clostridiales; unassigned Clostridiales															
6	ASV5	0	0	0	0	0	0	0	4	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium															
7	ASV6	649	803	506	616	922	770	978	114	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira															
8	ASV7	440	209	523	222	742	95	390	742	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
9	ASV8	796	871	572	990	861	915	633	330	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unassigned Lachnospiraceae															
10	ASV9	0	0	0	0	0	0	32	33	Bacteria; Proteobacteria; NA; NA; NA; NA															
11	ASV10	382	404	150	658	118	298	436	102	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; RF16; unassigned RF16															
12	ASV11	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; unassigned Prevotellaceae															
13	ASV12	868	357	167	917	96	810	107	75	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; unassigned S24-7															
14	ASV13	294	121	693	645	359	729	130	510	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; BF311															
15	ASV14	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; unassigned Bacteroidales; unassigned Bacteroidales															
16	ASV15	0	0	0	0	0	0	5	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; CF231															
17	ASV16	0	0	66	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella															
18	ASV17	0	0	0	0	6	0	4	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; unassigned [Paraprevotellaceae]															
19	ASV18	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; YRC22															
20	ASV19	999	962	896	555	977	710	771	700	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; BS11; unassigned BS11															
21	ASV20	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; [Weeksellaceae]; unassigned [Weeksellaceae]															
22	ASV21	0	0	0	0	0	0	0	0	Bacteria; Verrucomicrobia; Verruco-5; WCHB1-41; RFP12; unassigned RFP12															
23	ASV22	0	0	65	0	0	0	0	0	Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobrevibacter															
24	ASV23	0	0	0	0	0	0	0	0	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema															
25	ASV24	416	193	959	975	526	625	328	134	Bacteria; Spirochaetes; Spirochaetes; Sphaerochaetales; Sphaerochaetaceae; Sphaerochaeta															
26	ASV25	972	96	112	167	463	814	130	902	Bacteria; Proteobacteria; Deltaproteobacteria; GMD14H09; unassigned GMD14H09; unassigned GMD14H09															
27	ASV26	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio															
28	ASV27	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; Succinimonas															
29	ASV28	979	643	740	716	504	140	33	182	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; unassigned Succinivibrionaceae															
30	ASV29	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Succiniclasticum															
31	ASV30	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; unassigned Veillonellaceae															
32	ASV31	539	184	899	142	455	419	335	537	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Anaerovibrio															
33	ASV32	80								Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas															
34	ASV33	0	0	0	0	0	0	0	0	Bacteria; Acidobacteria; [Chloracidobacteria]; RB41; Ellin6075; unassigned Ellin6075															
35	ASV34	316	611	210	750	715	718	538	234	Bacteria; WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2															
36	ASV35	838	769	357	725	253	33	530	818	Bacteria; Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira															
37	ASV36	0	0	0	0	0	0	0	0	Bacteria; Fibrobacteres; Fibrobacteria; Fibrobacterales; Fibrobacteraceae; Fibrobacter															
38	ASV37	0	43	0	0	32	0	0	0	Bacteria; Planctomycetes; Planctomycetia; Pirellulales; Pirellulaceae; unassigned Pirellulaceae															
39	ASV38	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; unassigned [Mogibacteriaceae]															
40	ASV39	680	773	798	395	225	464	519	228	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; Mogibacterium															



Sparsity = lots of zeros

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1		Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	taxonomy															
2	ASV1	405	178	545	357	143	829	698	881	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio															
3	ASV2	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
4	ASV3	634	111	940	352	179	545	887	92	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; NA; NA															
5	ASV4	837	733	858	905	127	312	655	796	Bacteria; Firmicutes; Clostridia; Clostridiales; unassigned Clostridiales; unassigned Clostridiales															
6	ASV5	0	0	0	0	0	0	0	4	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium															
7	ASV6	649	803	506	616	922	770	978	114	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira															
8	ASV7	440	209	523	222	742	95	390	742	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
9	ASV8	796	871	572	990	861	915	633	330	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unassigned Lachnospiraceae															
10	ASV9	0	0	0	0	0	0	32	33	Bacteria; Proteobacteria; NA; NA; NA; NA															
11	ASV10	382	404	150	658	118	298	436	102	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; RF16; unassigned RF16															
12	ASV11	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; unassigned Prevotellaceae															
13	ASV12	868	357	167	917	96	810	107	75	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; unassigned S24-7															
14	ASV13	294	121	693	645	359	729	130	510	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; BF311															
15	ASV14	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; unassigned Bacteroidales; unassigned Bacteroidales															
16	ASV15	0	0	0	0	0	0	5	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; CF231															
17	ASV16	0	0	66	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella															
18	ASV17	0	0	0	0	0	6	0	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; unassigned [Paraprevotellaceae]															
19	ASV18	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; YRC22															
20	ASV19	999	962	896	555	977	710	771	700	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; BS11; unassigned BS11															
21	ASV20	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; [Weeksellaceae]; unassigned [Weeksellaceae]															
22	ASV21	0	0	0	0	0	0	0	0	Bacteria; Verrucomicrobia; Verruco-5; WCHB1-41; RFP12; unassigned RFP12															
23	ASV22	0	0	65	0	0	0	0	0	Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobrevibacter															
24	ASV23	0	0	0	0	0	0	0	0	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema															
25	ASV24	416	193	959	975	526	625	328	134	Bacteria; Spirochaetes; Spirochaetes; Sphaerochaetales; Sphaerochaetaceae; Sphaerochaeta															
26	ASV25	972	96	112	167	463	814	130	902	Bacteria; Proteobacteria; Deltaproteobacteria; GMD14H09; unassigned GMD14H09; unassigned GMD14H09															
27	ASV26	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio															
28	ASV27	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; Succinimonas															
29	ASV28	979	643	740	716	504	140	33	182	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; unassigned Succinivibrionaceae															
30	ASV29	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Succiniclasticum															
31	ASV30	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; unassigned Veillonellaceae															
32	ASV31	539	184	899	142	455	419	335	537	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Anaerovibrio															
33	ASV32	80								Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas															
34	ASV33	0	0	0	0	0	0	0	0	Bacteria; Acidobacteria; [Chloracidobacteria]; RB41; Ellin6075; unassigned Ellin6075															
35	ASV34	316	611	210	750	715	718	538	234	Bacteria; WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2															
36	ASV35	838	769	357	725	253	33	530	818	Bacteria; Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira															
37	ASV36	0	0	0	0	0	0	0	0	Bacteria; Fibrobacteres; Fibrobacteria; Fibrobacterales; Fibrobacteraceae; Fibrobacter															
38	ASV37	0	43	0	0	32	0	0	0	Bacteria; Planctomycetes; Planctomycetia; Pirellulales; Pirellulaceae; unassigned Pirellulaceae															
39	ASV38	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; unassigned [Mogibacteriaceae]															
40	ASV39	680	773	798	395	225	464	519	228	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; Mogibacterium															



Transformation of count data

- For some analyses, we can perform **Hellinger transformation** to convert taxa abundances from absolute to relative values (i.e. standardizes the abundances to sample totals) and then square roots them.
- This decreases the impact of highly abundant features
- Some research questions might require transformation to determine the presence/absence of each taxa

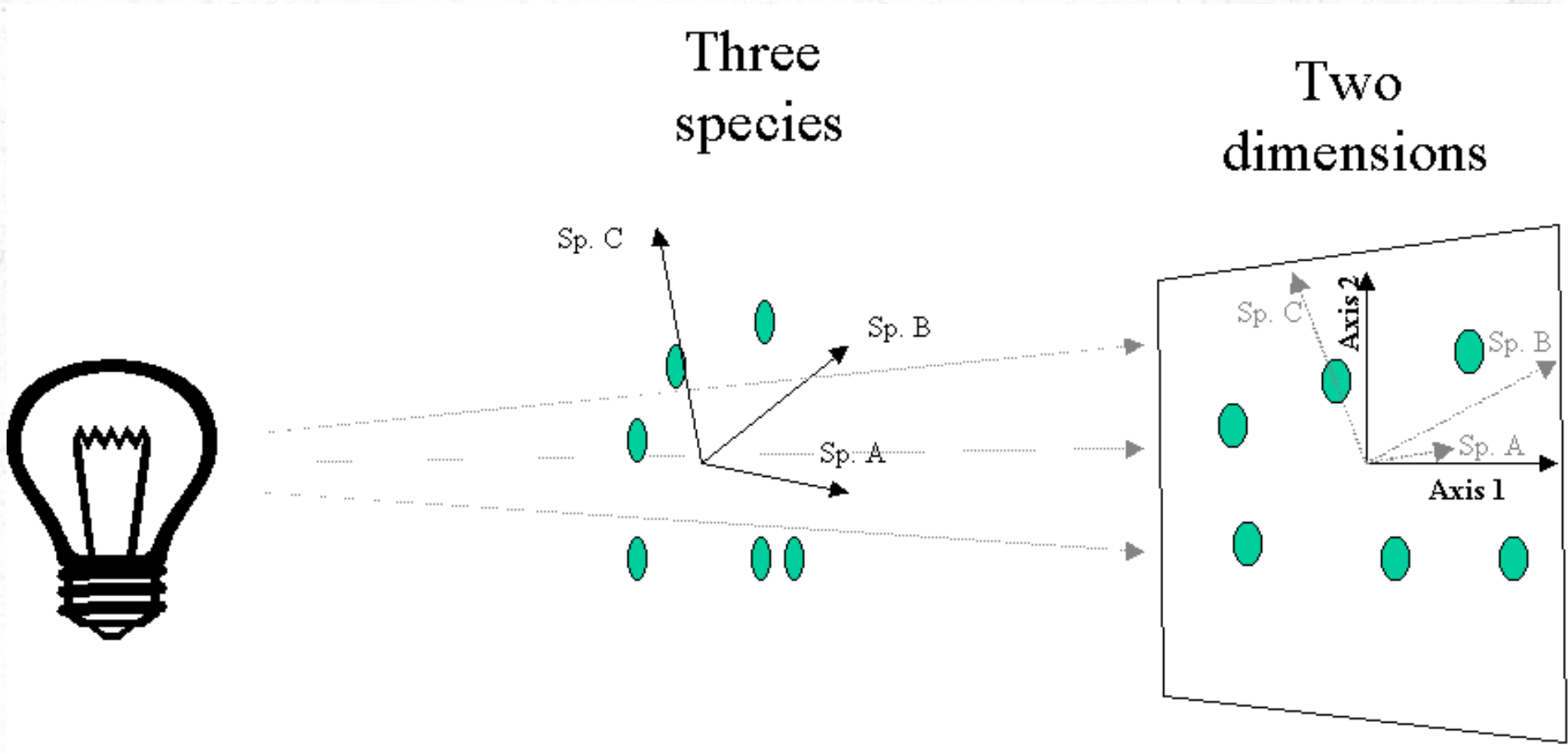


Multidimensional data - how to compare across 100s of taxa?

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1		Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	taxonomy															
2	ASV1	405	178	545	357	143	829	698	881	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio															
3	ASV2	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
4	ASV3	634	111	940	352	179	545	887	92	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; NA; NA															
5	ASV4	837	733	858	905	127	312	655	796	Bacteria; Firmicutes; Clostridia; Clostridiales; unassigned Clostridiales; unassigned Clostridiales															
6	ASV5	0	0	0	0	0	0	0	4	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium															
7	ASV6	649	803	506	616	922	770	978	114	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira															
8	ASV7	440	209	523	222	742	95	390	742	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
9	ASV8	796	871	572	990	861	915	633	330	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unassigned Lachnospiraceae															
10	ASV9	0	0	0	0	0	0	32	33	Bacteria; Proteobacteria; NA; NA; NA; NA															
11	ASV10	382	404	150	658	118	298	436	102	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; RF16; unassigned RF16															
12	ASV11	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; unassigned Prevotellaceae															
13	ASV12	868	357	167	917	96	810	107	75	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; unassigned S24-7															
14	ASV13	294	121	693	645	359	729	130	510	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; BF311															
15	ASV14	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; unassigned Bacteroidales; unassigned Bacteroidales															
16	ASV15	0	0	0	0	0	0	5	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; CF231															
17	ASV16	0	0	66	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella															
18	ASV17	0	0	0	0	6	0	4	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; unassigned [Paraprevotellaceae]															
19	ASV18	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; YRC22															
20	ASV19	999	962	896	555	977	710	771	700	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; BS11; unassigned BS11															
21	ASV20	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; [Weeksellaceae]; unassigned [Weeksellaceae]															
22	ASV21	0	0	0	0	0	0	0	0	Bacteria; Verrucomicrobia; Verruco-5; WCHB1-41; RFP12; unassigned RFP12															
23	ASV22	0	0	65	0	0	0	0	0	Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobrevibacter															
24	ASV23	0	0	0	0	0	0	0	0	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema															
25	ASV24	416	193	959	975	526	625	328	134	Bacteria; Spirochaetes; Spirochaetes; Sphaerochaetales; Sphaerochaetaceae; Sphaerochaeta															
26	ASV25	972	96	112	167	463	814	130	902	Bacteria; Proteobacteria; Deltaproteobacteria; GMD14H09; unassigned GMD14H09; unassigned GMD14H09															
27	ASV26	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio															
28	ASV27	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; Succinimonas															
29	ASV28	979	643	740	716	504	140	33	182	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; unassigned Succinivibrionaceae															
30	ASV29	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Succiniclasticum															
31	ASV30	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; unassigned Veillonellaceae															
32	ASV31	539	184	899	142	455	419	335	537	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Anaerovibrio															
33	ASV32	80								Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas															
34	ASV33	0	0	0	0	0	0	0	0	Bacteria; Acidobacteria; [Chloracidobacteria]; RB41; Ellin6075; unassigned Ellin6075															
35	ASV34	316	611	210	750	715	718	538	234	Bacteria; WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2															
36	ASV35	838	769	357	725	253	33	530	818	Bacteria; Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira															
37	ASV36	0	0	0	0	0	0	0	0	Bacteria; Fibrobacteres; Fibrobacteria; Fibrobacterales; Fibrobacteraceae; Fibrobacter															
38	ASV37	0	43	0	0	32	0	0	0	Bacteria; Planctomycetes; Planctomycetia; Pirellulales; Pirellulaceae; unassigned Pirellulaceae															
39	ASV38	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; unassigned [Mogibacteriaceae]															
40	ASV39	680	773	798	395	225	464	519	228	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; Mogibacterium															



Simplified example



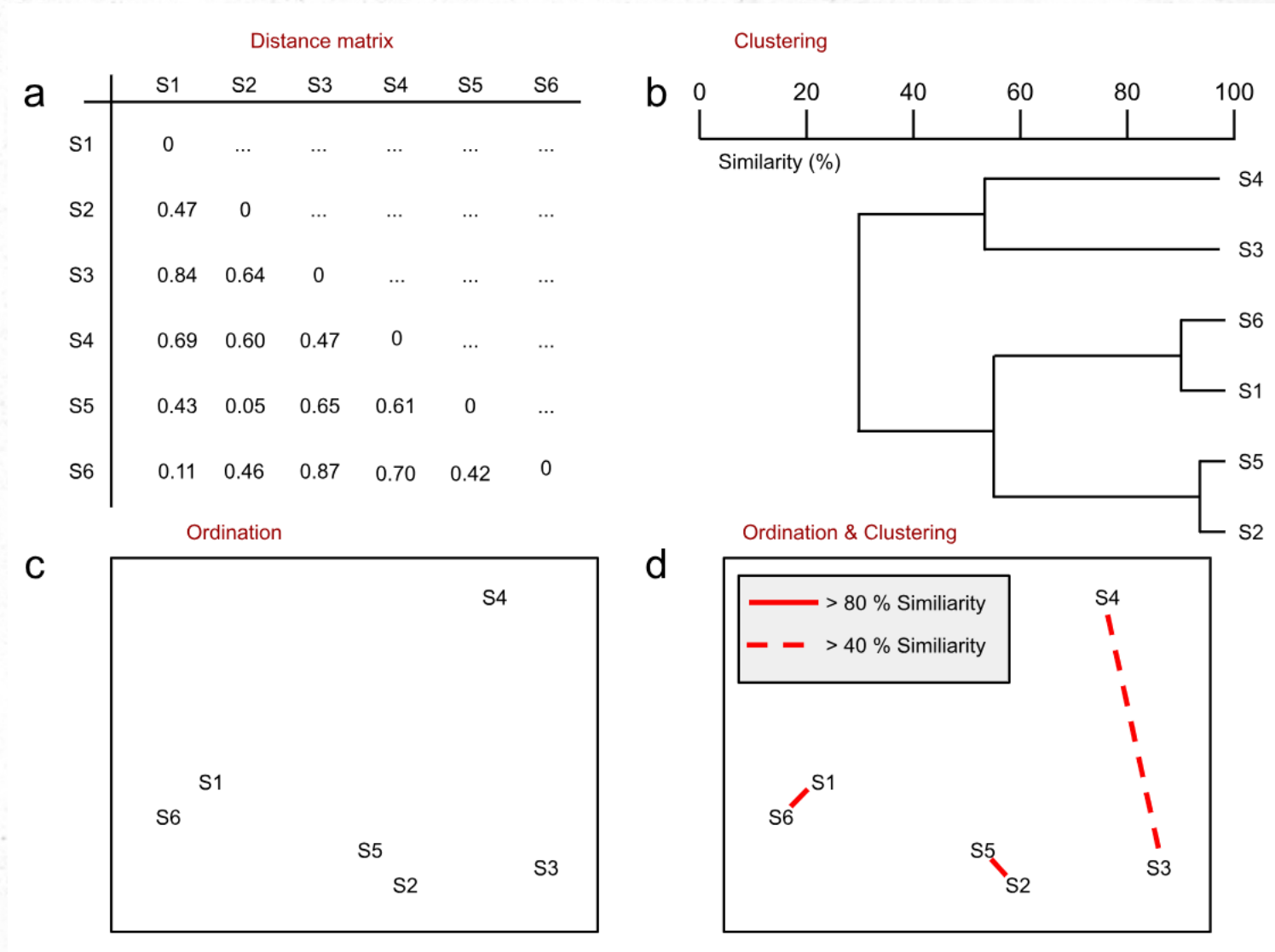
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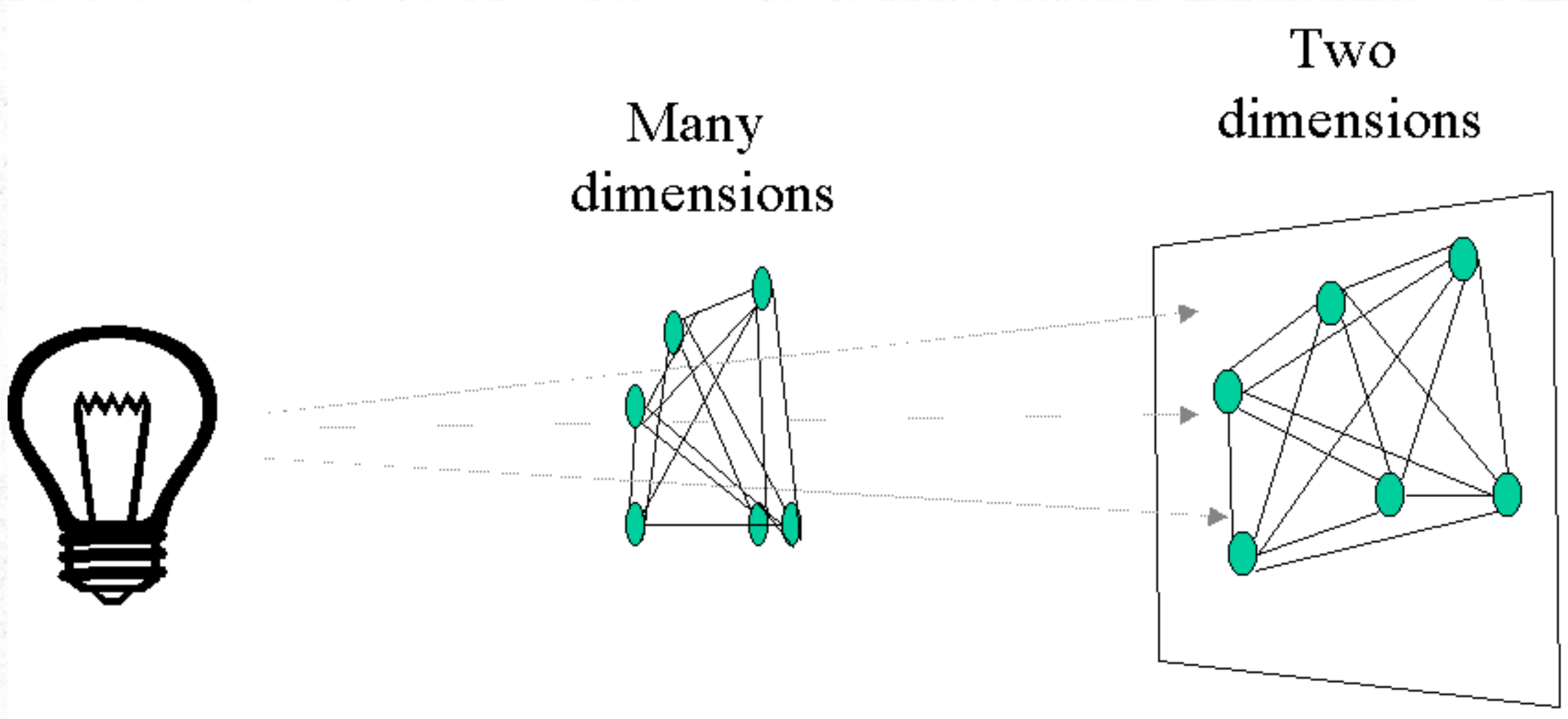
<https://ordination.okstate.edu/overview.htm>

Dimension reduction - Ordination

- We can use dissimilarity indices (beta diversity) and ordination to **visualize** the differences between all samples
- Then, we can use ordination to view results in 2 dimensions
- **Testing is not done on ordination**



More realistically, with distances between samples



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<https://ordination.okstate.edu/overview.htm>

Compositional = counts are out of 100% for each sample

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
1		Sample1	Sample2	Sample3	Sample4	Sample5	Sample6	Sample7	Sample8	taxonomy															
2	ASV1	405	178	545	357	143	829	698	881	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; Vibrionaceae; Vibrio															
3	ASV2	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
4	ASV3	634	111	940	352	179	545	887	92	Bacteria; Proteobacteria; Gammaproteobacteria; Vibrionales; NA; NA															
5	ASV4	837	733	858	905	127	312	655	796	Bacteria; Firmicutes; Clostridia; Clostridiales; unassigned Clostridiales; unassigned Clostridiales															
6	ASV5	0	0	0	0	0	0	0	4	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; Clostridium															
7	ASV6	649	803	506	616	922	770	978	114	Bacteria; Firmicutes; Clostridia; Clostridiales; Ruminococcaceae; Oscillospira															
8	ASV7	440	209	523	222	742	95	390	742	Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae; 02d06															
9	ASV8	796	871	572	990	861	915	633	330	Bacteria; Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; unassigned Lachnospiraceae															
10	ASV9	0	0	0	0	0	0	32	33	Bacteria; Proteobacteria; NA; NA; NA; NA															
11	ASV10	382	404	150	658	118	298	436	102	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; RF16; unassigned RF16															
12	ASV11	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; unassigned Prevotellaceae															
13	ASV12	868	357	167	917	96	810	107	75	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; S24-7; unassigned S24-7															
14	ASV13	294	121	693	645	359	729	130	510	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Bacteroidaceae; BF311															
15	ASV14	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; unassigned Bacteroidales; unassigned Bacteroidales															
16	ASV15	0	0	0	0	0	0	5	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; CF231															
17	ASV16	0	0	66	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; Prevotellaceae; Prevotella															
18	ASV17	0	0	0	0	0	6	0	4	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; unassigned [Paraprevotellaceae]															
19	ASV18	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; [Paraprevotellaceae]; YRC22															
20	ASV19	999	962	896	555	977	710	771	700	Bacteria; Bacteroidetes; Bacteroidia; Bacteroidales; BS11; unassigned BS11															
21	ASV20	0	0	0	0	0	0	0	0	Bacteria; Bacteroidetes; Flavobacteriia; Flavobacteriales; [Weeksellaceae]; unassigned [Weeksellaceae]															
22	ASV21	0	0	0	0	0	0	0	0	Bacteria; Verrucomicrobia; Verruco-5; WCHB1-41; RFP12; unassigned RFP12															
23	ASV22	0	0	65	0	0	0	0	0	Archaea; Euryarchaeota; Methanobacteria; Methanobacteriales; Methanobacteriaceae; Methanobrevibacter															
24	ASV23	0	0	0	0	0	0	0	0	Bacteria; Spirochaetes; Spirochaetes; Spirochaetales; Spirochaetaceae; Treponema															
25	ASV24	416	193	959	975	526	625	328	134	Bacteria; Spirochaetes; Spirochaetes; Sphaerochaetales; Sphaerochaetaceae; Sphaerochaeta															
26	ASV25	972	96	112	167	463	814	130	902	Bacteria; Proteobacteria; Deltaproteobacteria; GMD14H09; unassigned GMD14H09; unassigned GMD14H09															
27	ASV26	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio															
28	ASV27	0	0	0	0	0	0	0	0	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; Succinimonas															
29	ASV28	979	643	740	716	504	140	33	182	Bacteria; Proteobacteria; Gammaproteobacteria; Aeromonadales; Succinivibrionaceae; unassigned Succinivibrionaceae															
30	ASV29	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Succinoclasticum															
31	ASV30	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; unassigned Veillonellaceae															
32	ASV31	539	184	899	142	455	419	335	537	Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Anaerovibrio															
33	ASV32	80								Bacteria; Firmicutes; Clostridia; Clostridiales; Veillonellaceae; Selenomonas															
34	ASV33	0	0	0	0	0	0	0	0	Bacteria; Acidobacteria; [Chloracidobacteria]; RB41; Ellin6075; unassigned Ellin6075															
35	ASV34	316	611	210	750	715	718	538	234	Bacteria; WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2; unassigned WPS-2															
36	ASV35	838	769	357	725	253	33	530	818	Bacteria; Nitrospirae; Nitrospira; Nitrospirales; Nitrospiraceae; Nitrospira															
37	ASV36	0	0	0	0	0	0	0	0	Bacteria; Fibrobacteres; Fibrobacteria; Fibrobacterales; Fibrobacteraceae; Fibrobacter															
38	ASV37	0	43	0	0	32	0	0	0	Bacteria; Planctomycetes; Planctomycetia; Pirellulales; Pirellulaceae; unassigned Pirellulaceae															
39	ASV38	0	0	0	0	0	0	0	0	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; unassigned [Mogibacteriaceae]															
40	ASV39	680	773	798	395	225	464	519	228	Bacteria; Firmicutes; Clostridia; Clostridiales; [Mogibacteriaceae]; Mogibacterium															



Modeling sequencing count data for differential abundance

- “Count data”
 - Count data is typically modelled with a Poisson distribution
 - Sequencing count data is different

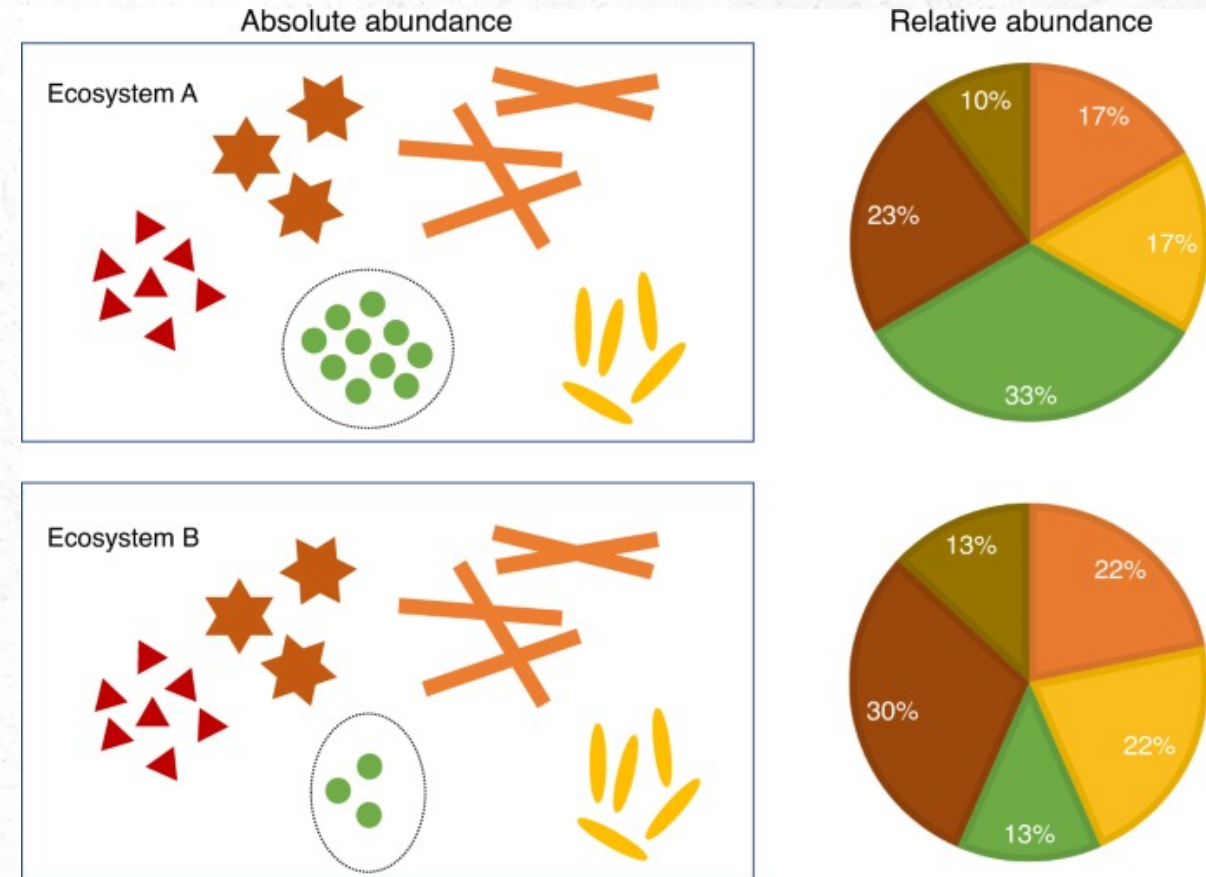
Microbiome Datasets Are Compositional: And This Is Not Optional

1. **Zero-inflated/sparse**

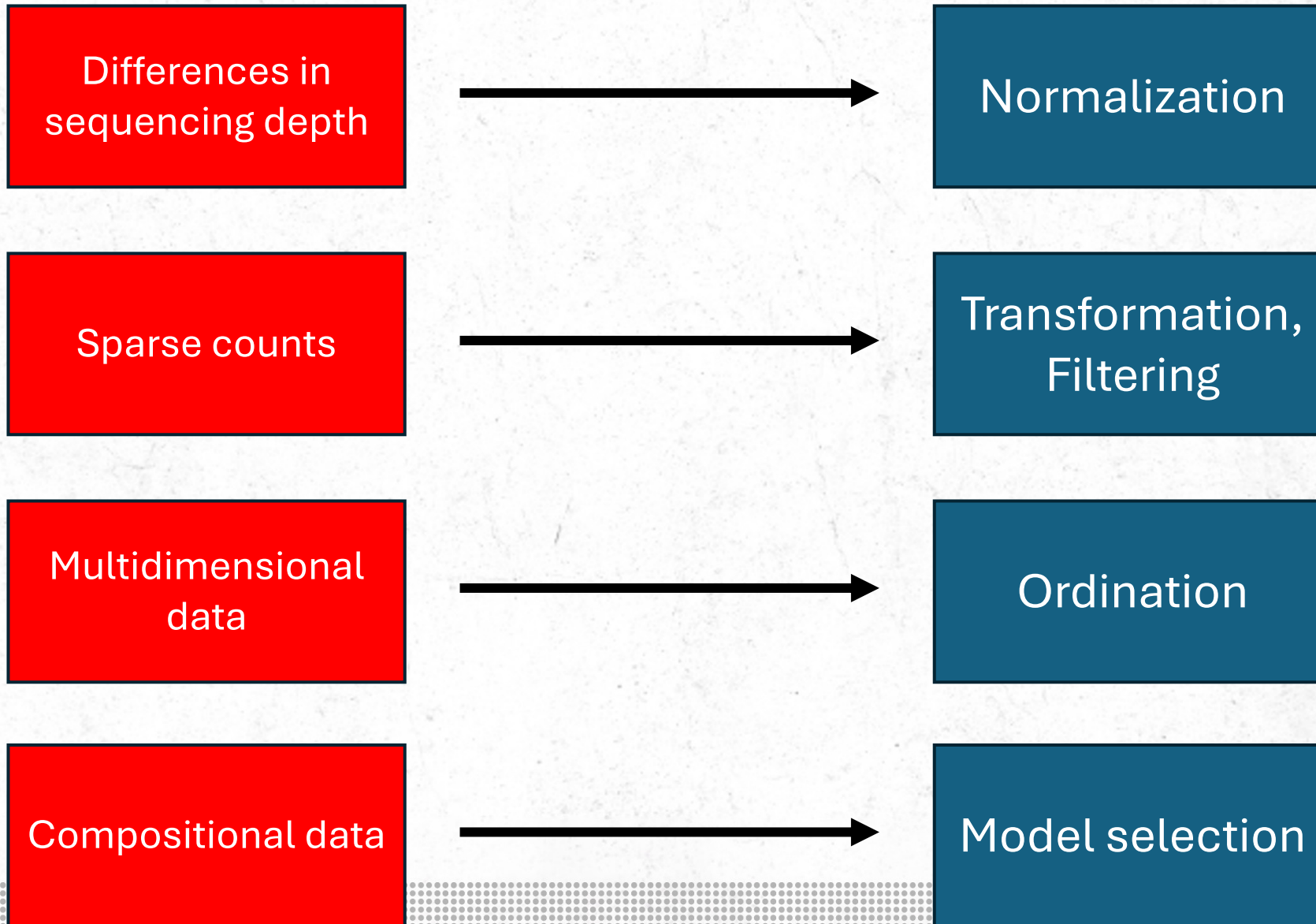
- Lots of features have 0's

2. **Compositional** due to a finite amount of sequencing

- Changes in one taxa, affect the others



What strategies can we employ?



Thoughts on interpreting results from multiple tests

- Each test we run contributes to our analyses and tells us something slightly different about our data
- It's important to keep in mind what your research question is and what tests help you get there.
- For example:
 - Does antimicrobial treatment affect microbiome composition = ordination
 - Which taxa were affected by antimicrobial treatment = differential abundance

