Invasive Vegetation affects amphibian skin microbiota and body condition

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What is the main question the authors want to address in this study?

Invasive Plant Species

- Landscape structure and configuration can have a major impact on how individuals move and how species spread.
- Non-native species tend to be invasive in an ecosystem.
- Freed from the constraints that limited their populations in their native range (predators, parasites, and competitors).
- Invasive species compete directly with native species for moisture, sunlight, nutrients, and space.
- Non-Native plant species spread in a landscape
  - Anthropogenic activity
  - Birds
  - Wind

Eucalyptus globulus

- Native to Australia and Tasmania
- Grown commercially for their oil and leaves.
- Thrive in areas where the temperature stays around 60 degrees Fahrenheit.
- *Eucalyptus* is an invasive species in California.
- *Eucalyptus* leaves can alter soil chemistry and negatively affect underground macro- and microbial communities (Hernández-Gómez et al. 2020).
    - Macrobial- species per area
    - Microbial- number of taxa (ex OTUs) in a sample that provide an estimate of diversity
- *Eucalyptus* decreases soil fertility
    - Degrades soil
    - Decreases soil pH
    - Increase soil acidity

Quercus agriflolia

- Common name: California live oak or Coast live oak
- Native to California
- Thrives in coastal environment
- Drought-resistant evergreen tree
- Range in height from 19 to 82 feet
- Fire resistant
    - Adaptations to fire include evergreen leaves, thick bark, and sprouting ability.

Batrachoseps attenuatus- California slender salamander

•Lungless salamander

•elongated and slender, with small, very short limbs and a long tail

•Each foot has 4 digits.

•Found primarily in coastal mountain areas of Northern California and Northern Sierra Nevada

•Found in several plant communities including California Oak woodland

•Occurrence ranges from valley floors to mid-elevation in coastal ranges.

•Usually found resting beneath leaf litter or other woodland detritus, or beneath rotting logs or rocks providing a wet environment.

salamander video

Why were the authors interested in *Eucalyptus*, and why did they focus on amphibian skin microbiota?

Methods (Molecular Ecology)

Are changes in microbial composition, diversity and stability in both **soil** and *B. attenuatus* **skin** associated with *Eucalyptus* or *Quercus* dominated habitats?

1. Study Area
2. Capture Mark Release Recapture
3. Quantitative PCR
4. Alignment
5. Quantify Diversity
6. Statistical Analysis

1. Study Area

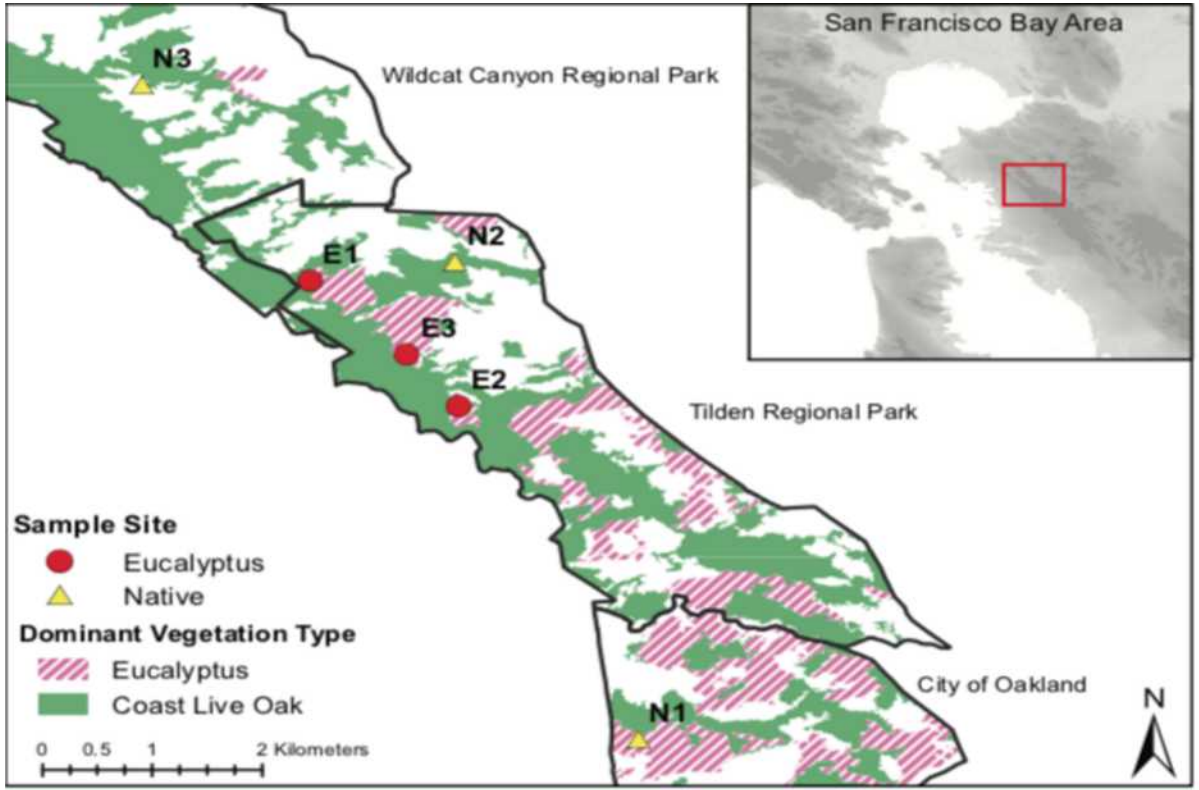
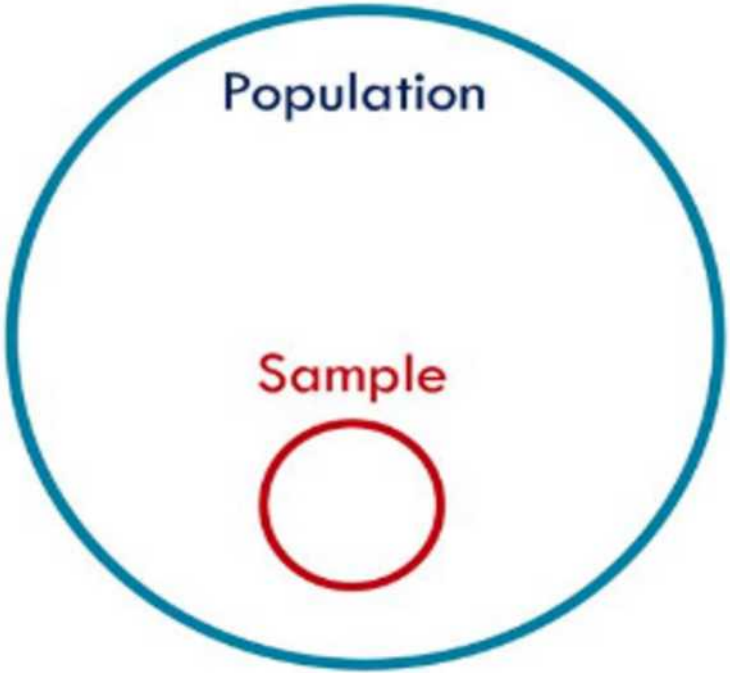


Figure 1 Dominant vegetation map of Wildcat Canyon Regional Park, Tilden Regional Park and UC Berkeley Campus. Sampling sites are displayed for *Quercus* and *Eucalyptus* dominant habitats from which *Batrachoseps attenuatus* skin microbiome swabs and soil were collected.

Full-size DOI: 10.7717/peerj.8549/fig-1

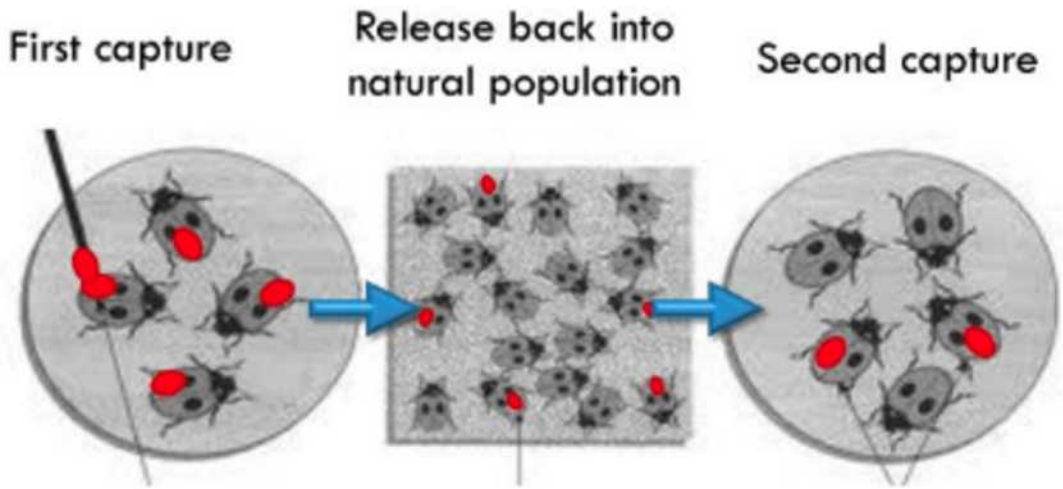
Population and Sample Size

- An efficient way to accurately estimate population size with minimal effort.
- Not feasible to count every individual.



2. Capture Mark Release Recapture (CMRR)

- Actually "capture-mark-release-recapture".
- Important when sampling mobile organisms.



CMRR Equation

$$N = Mn / m$$

Estimate actual population size from a sample subset.

N = population estimate

M = marked individuals in the first sample

n = total individuals in the second sample

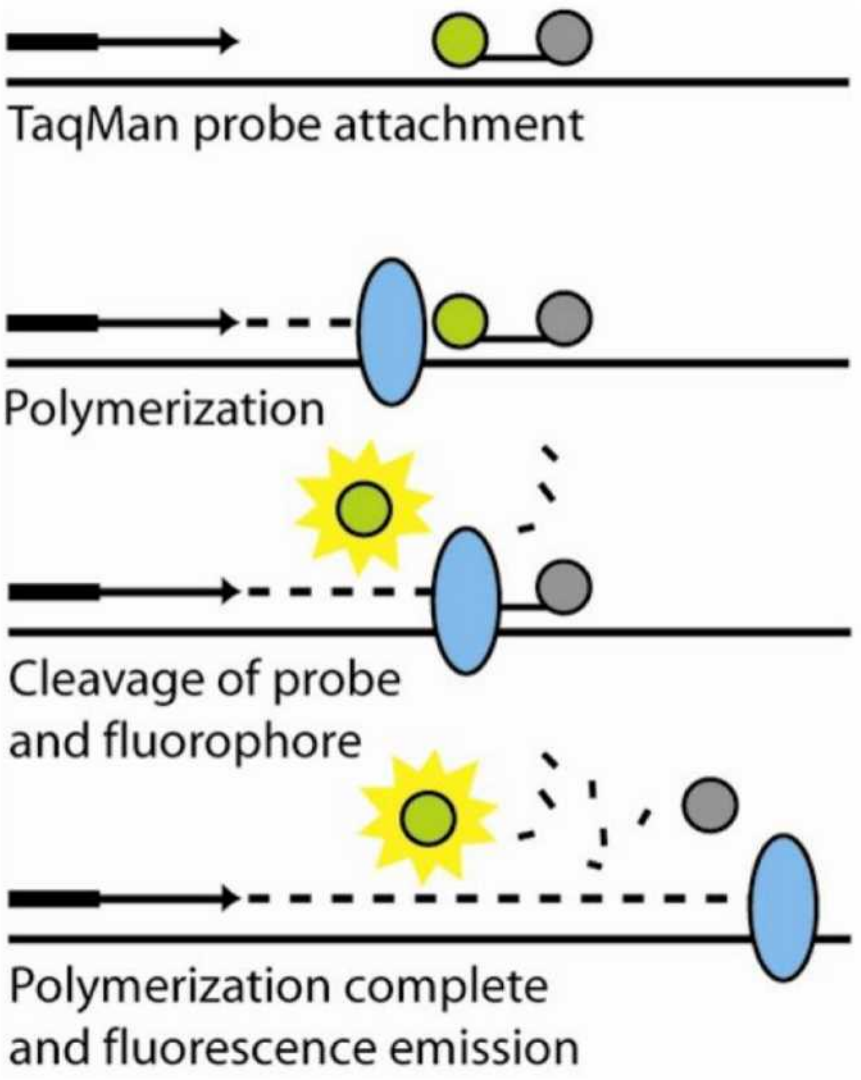
m = marked individuals captured in second sample

3. q-PCR

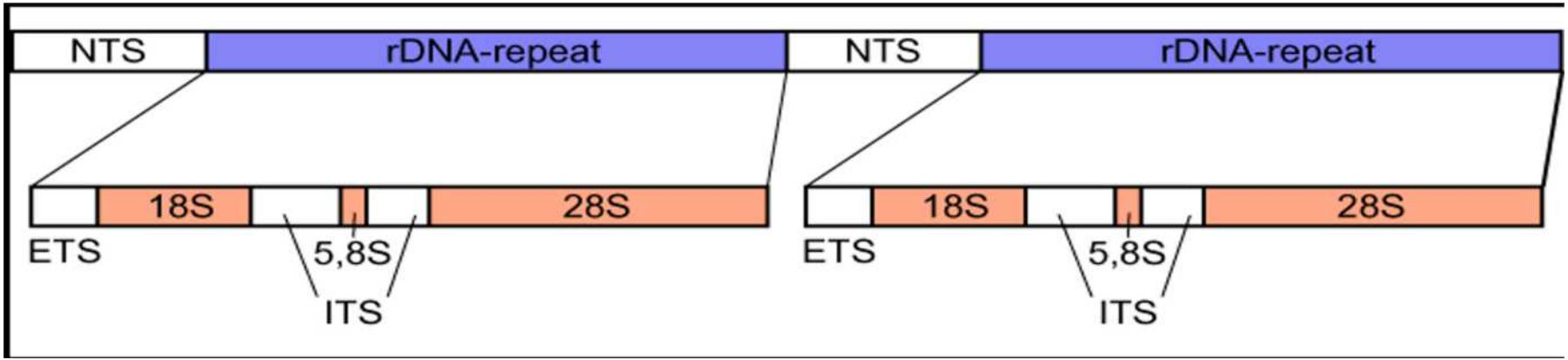
Fluorogenic 5’ nuclease assay

Taq polymerase

Quantitatively measure amplicons of DNA with greater specificity.

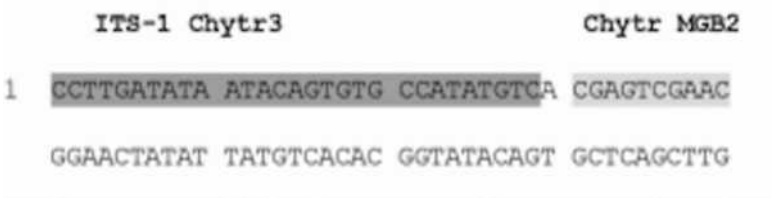


Eukaryotic Nuclear Ribosomal Cluster

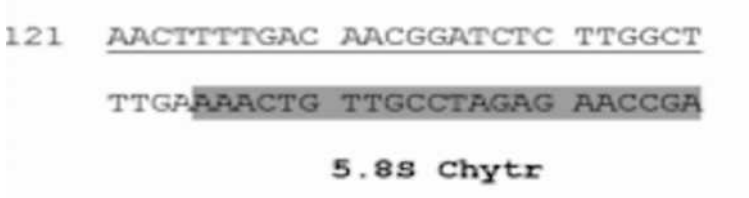


Internal Transcribed Spacer (ITS)
Discrete
Universal Fungal barcode sequence

rDNA sequence of *B. dendrobatidis* (Boyle et al. 2004)



ITS- 5.8S Junction rRNA



Primer

b

Primer Sequences

ITS1-3 Chytr	29 bases
5'- CCTTGATATAATACAGTGTGCCATATGTC-3'	
5.8S Chytr	22 bases
5'- AGCCAAGAGATCCGTTGTCAAA -3'	

MGB

Minor groove binder probe sequence

Chytr MGB2	15 bases
5' - 6FAM CGAGTCGAACAAAAT MGBNFQ – 3'	

Why quantify *Bd* using q-PCR?

From Longo et. al 2013:

[3]. *Bd* detection via qPCR has allowed researchers to detect infection levels in natural populations at different stages of emerging epidemics [4], track outbreaks that cause amphibian declines [5], establish disease thresholds predicting frog mortality [6], and reconstruct historical *Bd* epizootic waves spreading through naïve populations [7].

Why is it important to understand the mechanism of how *Bd* spreads?

Why create zoospore standards?

From Longo et al. 2013:

To generate standards for quantification of *Bd* via qPCR, researchers count zoospores from cultured *Bd* strains, extract genomic DNA (gDNA), and serially dilute to the desired concentrations (usually 100 to 0.1 zoospore genomic equivalents [2], [3]). The forward primer/probe combination of the qPCR TaqMan assay anneals to the internal transcribed spacer (ITS1) region, which is a rapidly evolving nuclear ribosomal repeat unit used for species-level identification [3], [11]. In fungal genomes, this region occurs in multiple copies providing over 100 potential primer/probe binding sites per haploid genome [3] and

Why is it important to understand Fungal Genomics to control the spread of *Bd?*

Amplicon Sequence Variants (ASVs)

OPEN

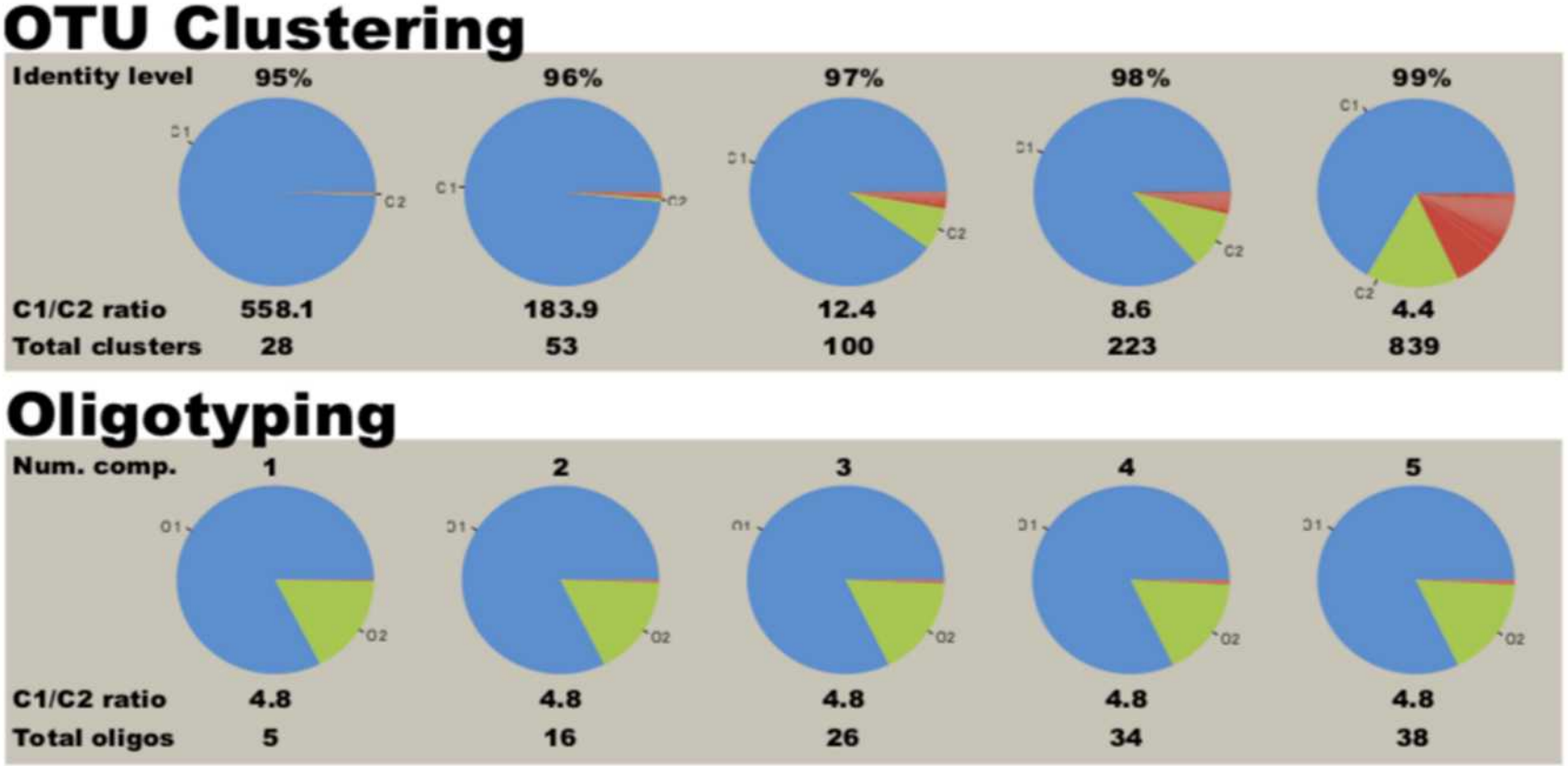
The ISME Journal (2017), 1–5
www.nature.com/ismej

PERSPECTIVE

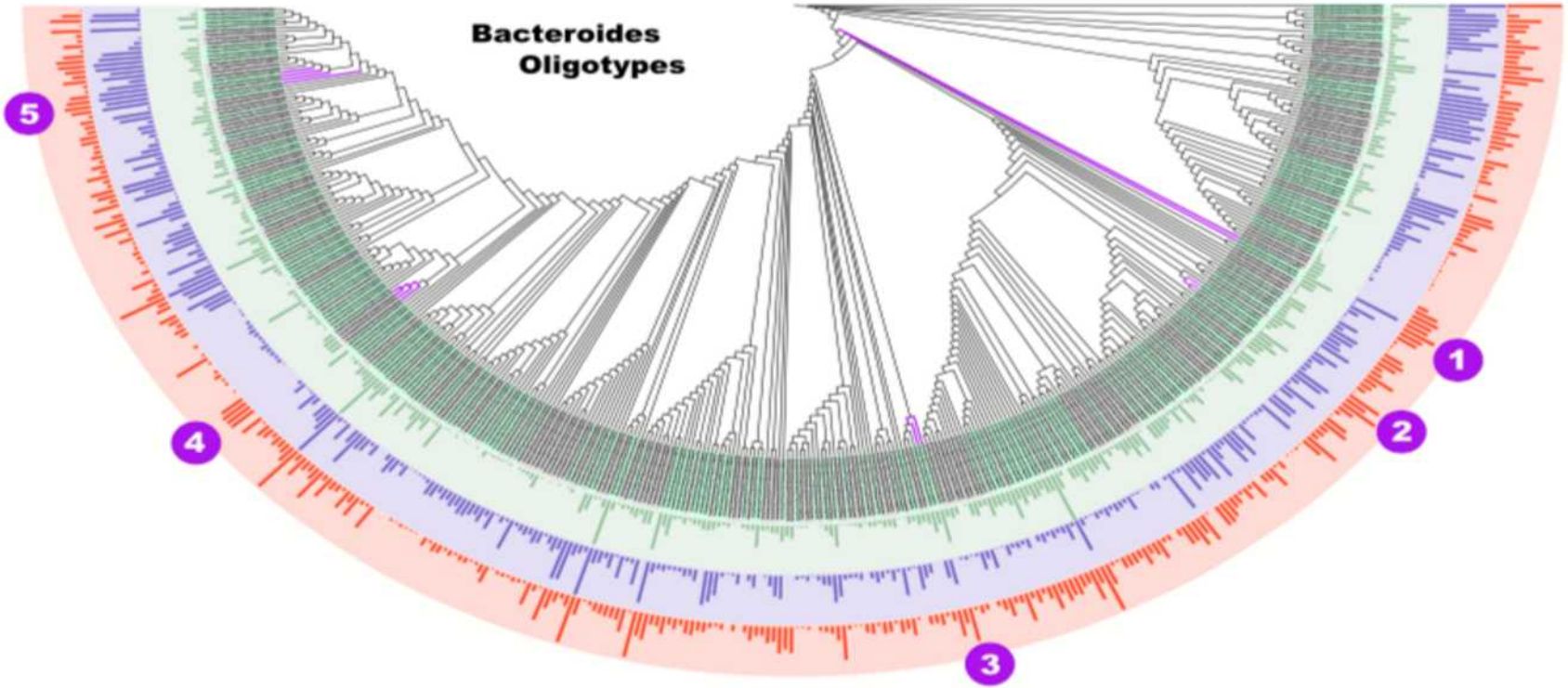
Exact sequence variants should replace operational taxonomic units in marker-gene data analysis

Benjamin J Callahan[1], Paul J McMurdie[2] and Susan P Holmes[3]
[1]*Department of Population Health and Pathobiology, NC State University, Raleigh NC, USA;* [2]*Whole Biome Inc, San Francisco CA, USA and* [3]*Department of Statistics, Stanford University, Stanford CA, USA*

Eren et al. 2013



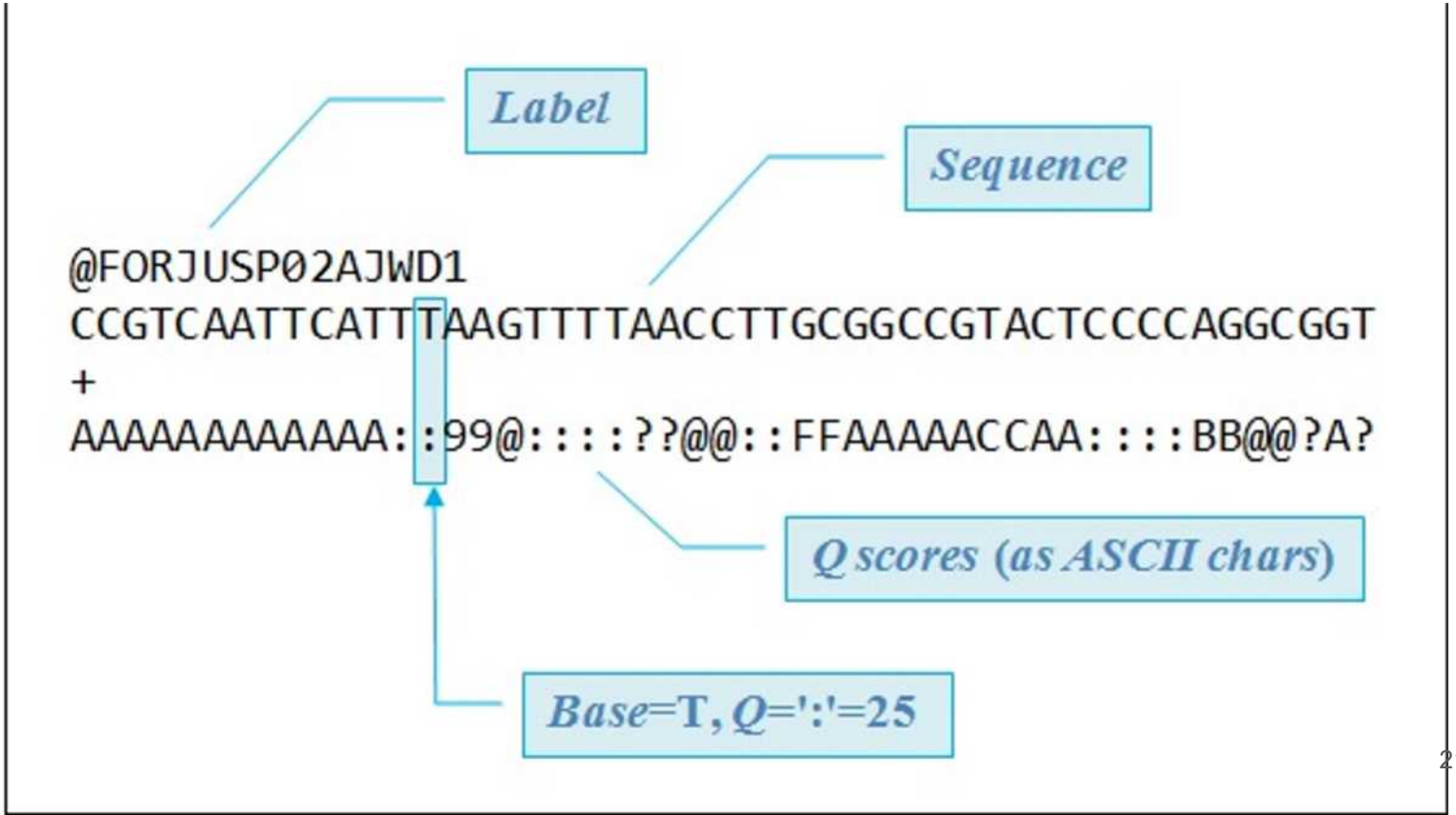
Eren et al. 2013



4. Alignment

FASTQ

ASCII



What is a q-score?

The quality score of a base, also known as a Phred or Q score, is an integer value representing the estimated probability of an error, i.e. that the base is incorrect. If P is the error probability, then:

$P = 10^{-Q/10}$

$Q = -10 \log_{10}(P)$

American Standard Code for Information exchange

128 characters into 0-7 bits

Q scores are often represented as ASCII characters. The rule for converting an ASCII character to an integer varies, see FASTQ options for details. Tables converting between integer Q scores, ASCII characters and error probabilities are shown in the table below ASCII_BASE 33, which is now almost universally used, and ASCII_BASE 64 which is used in some older Illumina data.

Phred (q-score) ASCII Table

Quality of base score estimated probability of error

ASCII_BASE=33 Illumina, Ion Torrent, PacBio and Sanger

Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII	Q	P_error	ASCII
0	1.00000	33 !	11	0.07943	44 ,	22	0.00631	55 7	33	0.00050	66 B
1	0.79433	34 "	12	0.06310	45 -	23	0.00501	56 8	34	0.00040	67 C
2	0.63096	35 #	13	0.05012	46 .	24	0.00398	57 9	35	0.00032	68 D
3	0.50119	36 $	14	0.03981	47 /	25	0.00316	58 :	36	0.00025	69 E
4	0.39811	37 %	15	0.03162	48 0	26	0.00251	59 ;	37	0.00020	70 F
5	0.31623	38 &	16	0.02512	49 1	27	0.00200	60 <	38	0.00016	71 G
6	0.25119	39 '	17	0.01995	50 2	28	0.00158	61 =	39	0.00013	72 H
7	0.19953	40 (	18	0.01585	51 3	29	0.00126	62 >	40	0.00010	73 I
8	0.15849	41)	19	0.01259	52 4	30	0.00100	63 ?	41	0.00008	74 J
9	0.12589	42 *	20	0.01000	53 5	31	0.00079	64 @	42	0.00006	75 K
10	0.10000	43 +	21	0.00794	54 6	32	0.00063	65 A			

Species-level identification- Supplemental S1

Assignment	ASV ID	LDA stats		*Eucalyptus* Sites			*Quercus* sites		
		LDA	p.value	E1	E2	E3	N1	N2	N3
	62ab3ec11e2c7d247ec90a010f08b127	0.56	0.025	0	1.3E-03	3.4E-04	0	7.3E-05	0
	1fad3b361787345c5c1012dbca74b747	0.52	0.035	0	9.8E-04	5.6E-04	0	0	0
	6424250710a6c8f3ecd0fd26603bcbdb	0.52	0.04	3.9E-04	0	3.2E-04	0	0	0
	959dc33fb13c310dd52835caa52eeca4	0.81	0.005	5.4E-04	6.7E-03	1.0E-02	5.4E-04	4.9E-05	0
	4066eea496dc43011db89efa755d95ba	0.52	0.04	0	9.1E-04	1.5E-03	0	0	0
	20b686910cf9db4b08f7b7c220800316	0.73	0.005	1.9E-03	7.3E-03	3.7E-03	1.0E-03	1.6E-03	0

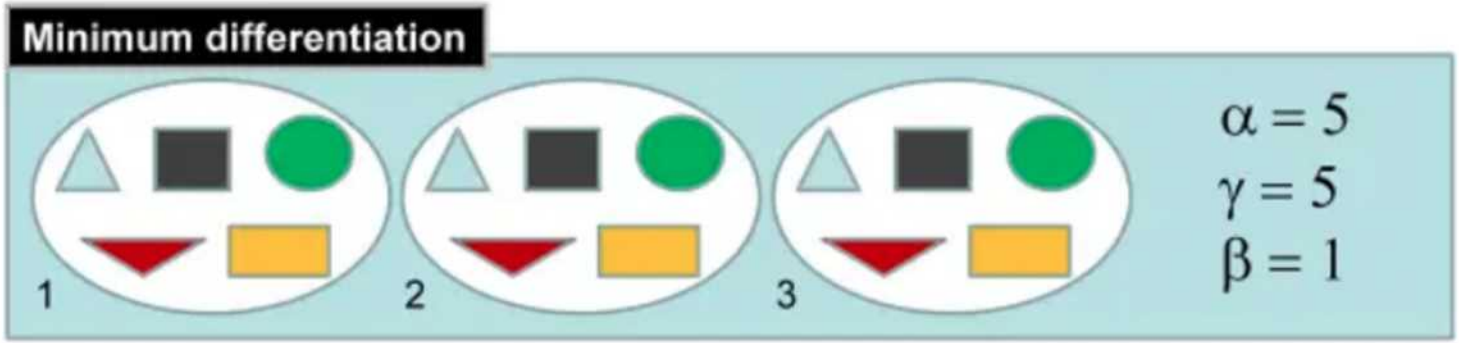
QIIME-2

LDA (Linear Discriminant Analysis)--reduction classifier

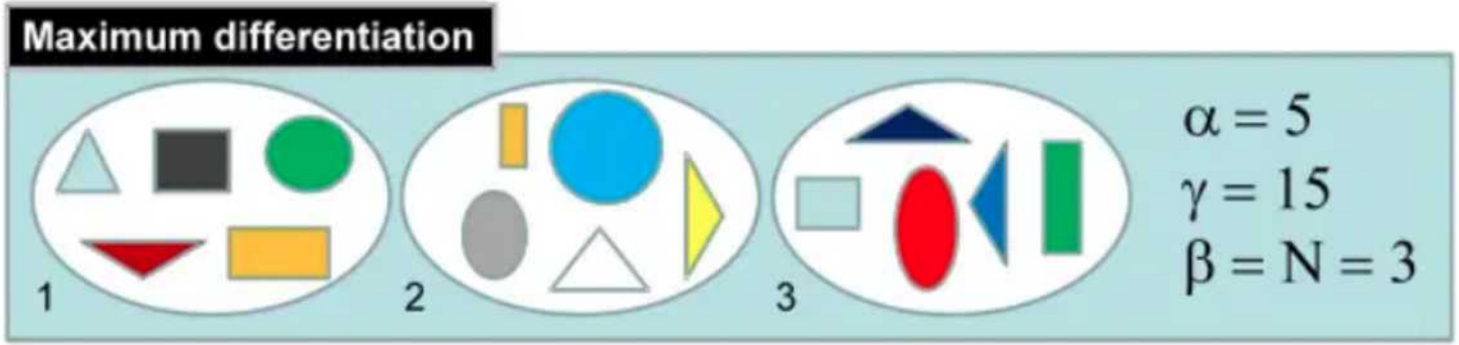
p-value

5. Quantify Diversity: Alpha and Beta

1.



2.



Why standardize (rarefy) alpha & beta diversity?

From Stier et al. 2016:

To minimize bias from sampling effects

The effects of patch size and sampling effort (which we collectively call *sampling effects*) are well known in studies of alpha diversity. Well-established tools, such as rarefaction or multinomial extrapolation, facilitate the comparison of alpha diversity among communities that differ in sampling protocols (e.g., number of samples, individuals, or coverage [fraction of the species list sampled]; Gotelli and

Alpha Diversity (within population) Metrics

Species richness (OTU count) "How many?"

How many different species could be detected in a microbial ecosystem?

DADA2 (QIIME-2) analysis
ASV count

Species diversity (Shannon index) "How different?"

How are the microbes balanced to each other? Do we have species evenness (similar abundance level) or do some species dominate others?

Shannon Index (evenness of distribution)
Faith's Phylogenetic Diversity (distance)

Beta Diversity (between population) Metrics

How different is the microbial composition in one environment compared to another?

Bray-Curtis Dissimilarity (abundance, 0 -1)
Unweighted Unifrac (sequence distances only)
Weighted Unifrac (sequence distances and abundance)

UniFrac: Unique Fraction Metric
Point-to-centroid distances

6. Statistical Analysis: Using beta metrics to predict microbial community composition

- Negative Binomial Error Distribution (richness)
- Likelihood Ratio Tests (chi-squared fit of two models)
- PERMANOVA (Permutational Multivariate Analysis of Variance -- Distance Matrix)
- NMDS plots (Non-metric multidimensional scaling -- Rank)
- Generalized Linear Mixed Models (randomness)
- Isolation by Distance (Mantel test- geographic sampling distances)

Using beta metrics to predict body index condition

- Least squares regression residuals (mass and total body length)
- Linear Mixed Model (randomness)
- Pearson Correlation (skin alpha diversity and body condition/energy reserves)

Figure 2. -Microhabitat Soil

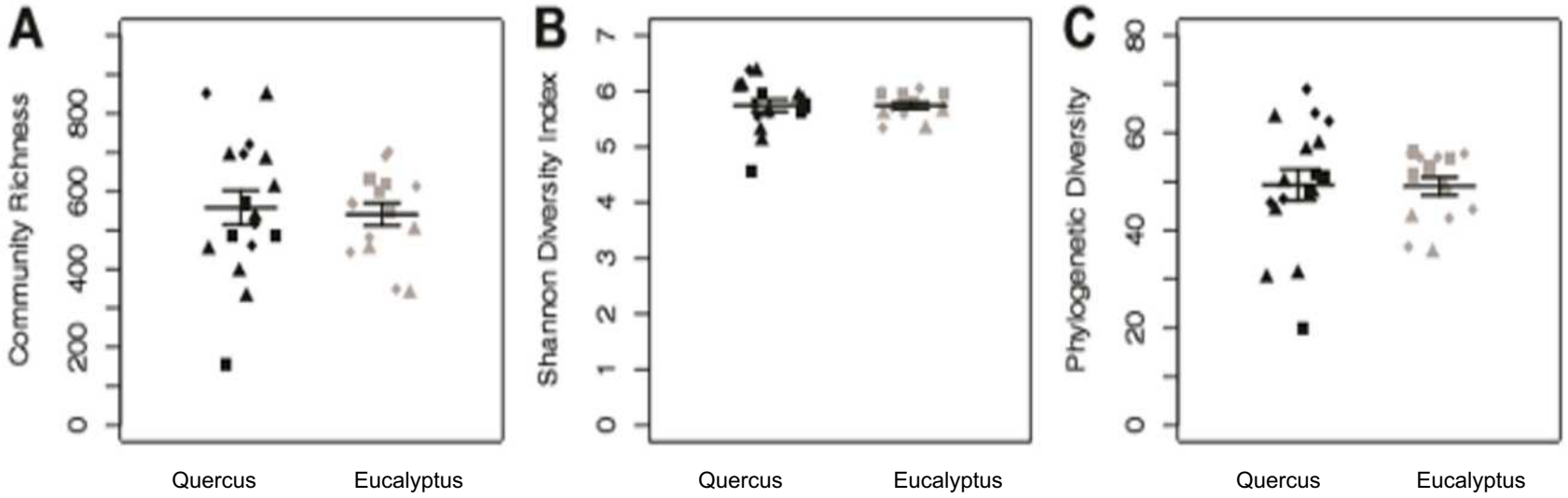


Figure 2.- *B. attenuatus* skin microbiota

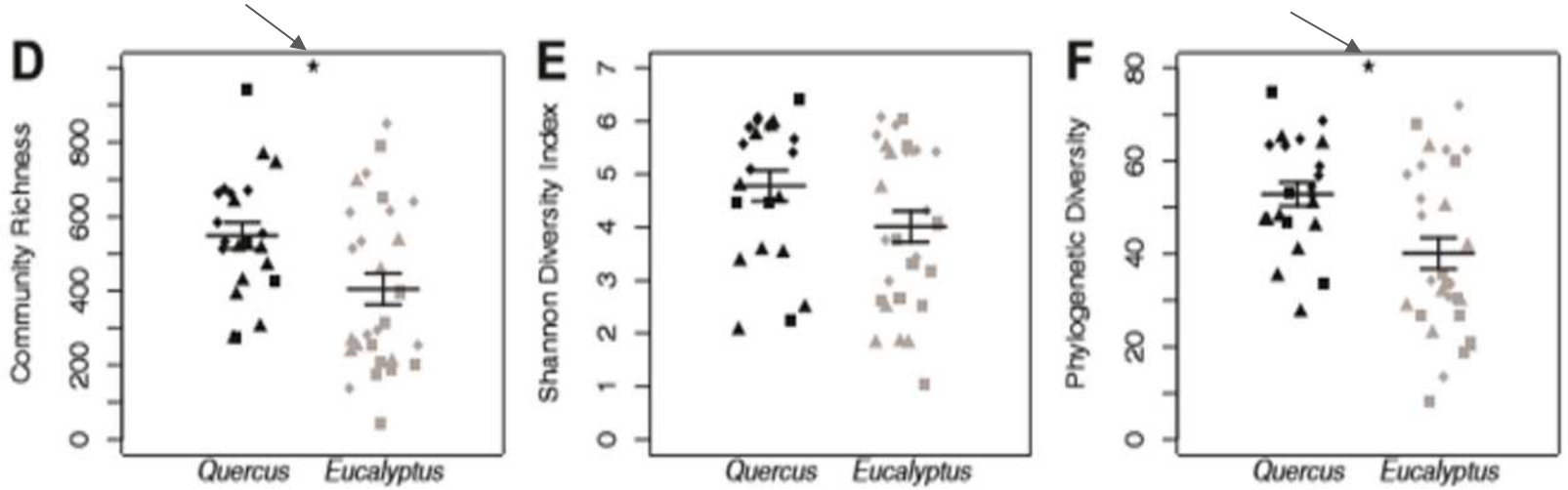


Figure 3.-Distance Matrices from microhabitat soils

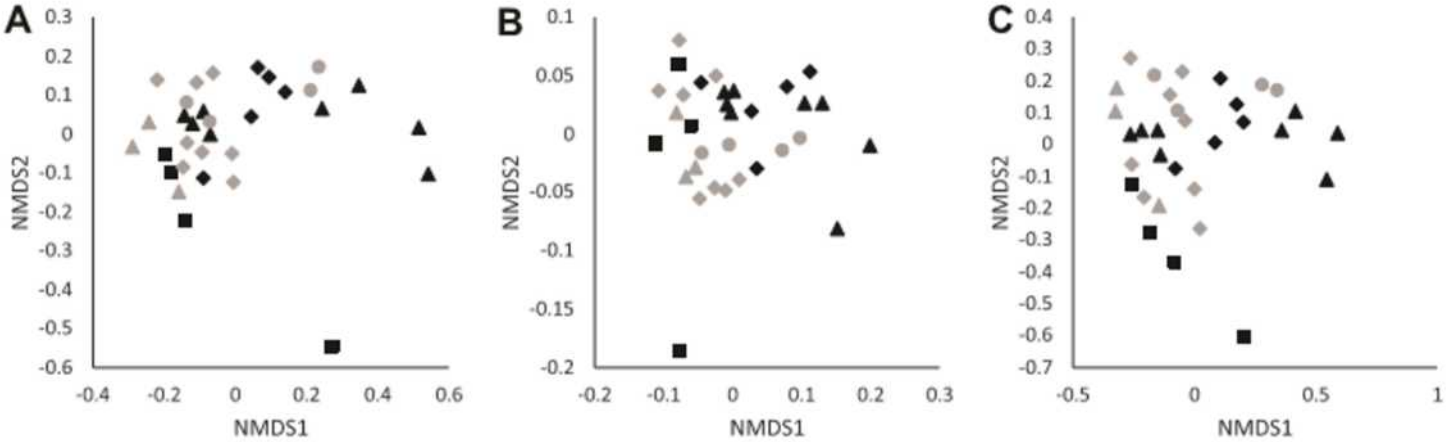


Figure 3.-Distance matrices for *B. attenuatus* skin microbiota sample

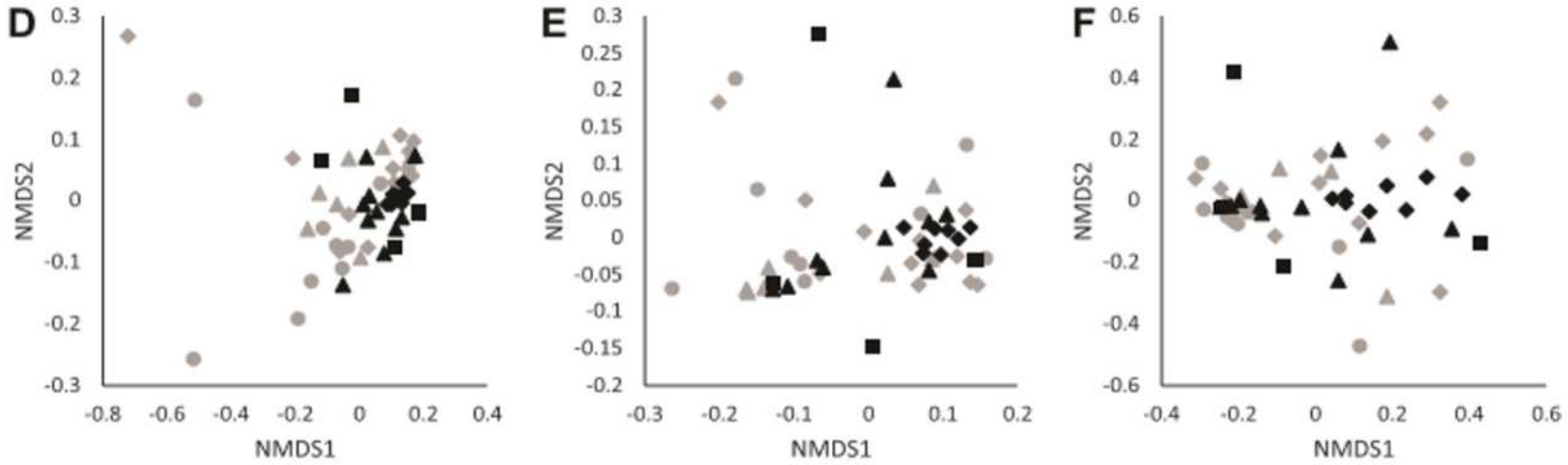


Figure 4-Betadisper of *B. attenuatus* skin microbiota samples collected in Quercus & Eucalyptus habitats

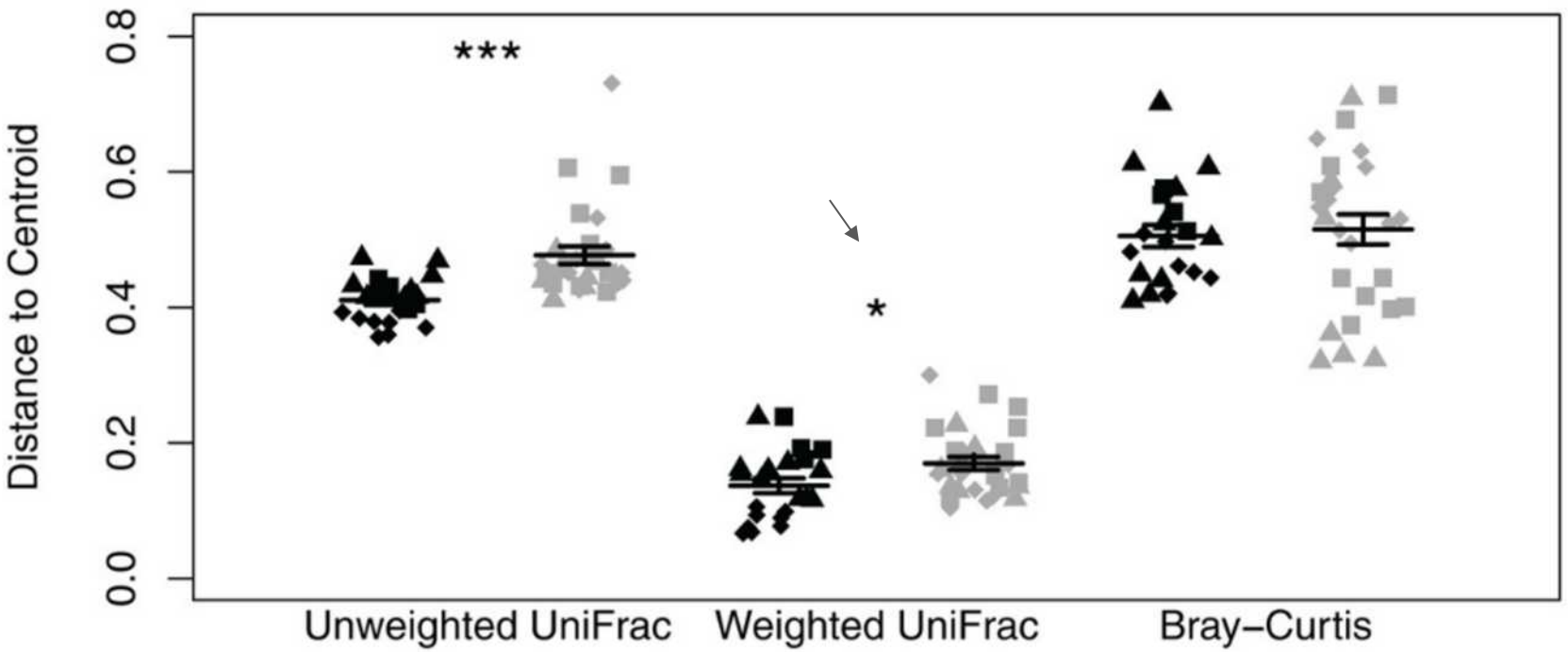


Figure 5.- *B. attenuatus* body condition index dot plot

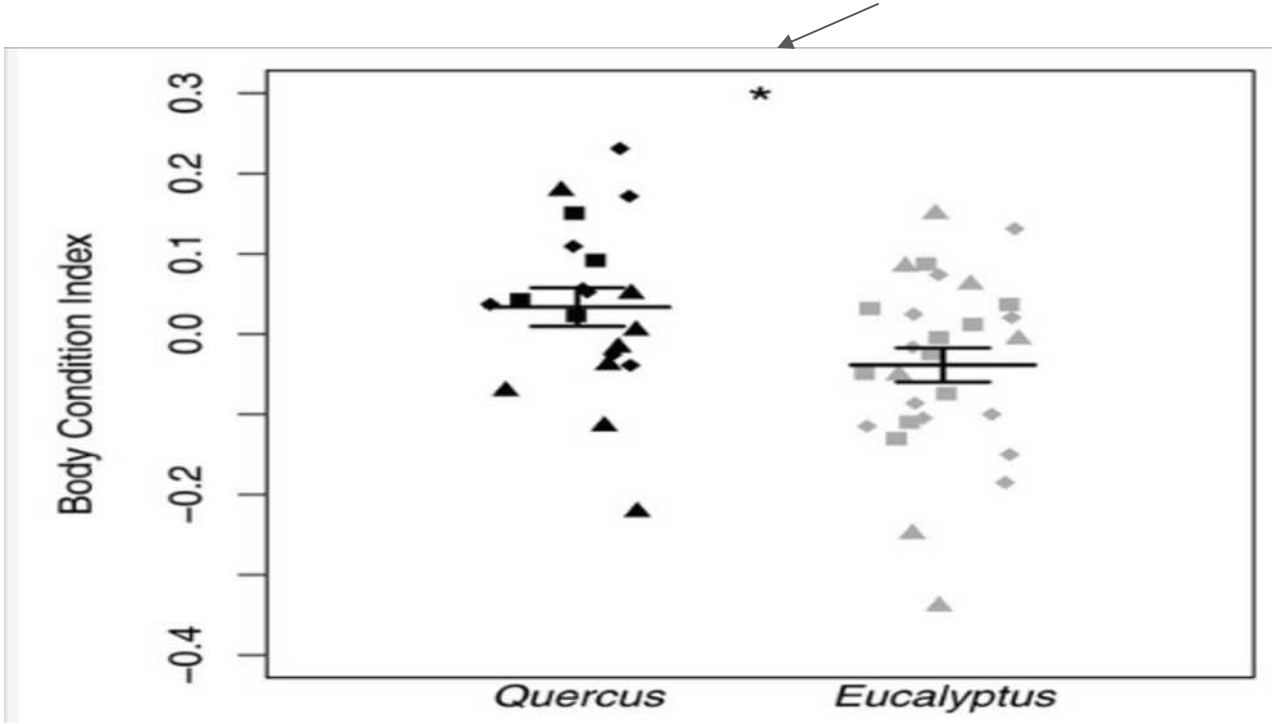


Table S1-B. attenuatus microhabitat soil microbiota indicator species analysis results.

		LDA stats		*Eucalyptus* Sites			*Quercus* sites			Taxonomy				
Assignment	ASV ID	LDA	p.value	E1	E2	E3	N1	N2	N3	Kingdom	Phylum	Class	Order	Family
	62ab3ec11e2c7d247ec90a010f08b127	0.56	0.025	0	1.3E-03	3.4E-04	0	7.3E-05	0	Archaea	Crenarchaeota	Thaumarchaeota	Nitrososphaerales	Nitrososphaeraceae
	1fad3b361787345c5c1012dbca74b747	0.52	0.035	0	9.8E-04	5.6E-04	0	0	0	Bacteria	Acidobacteria	Chloracidobacteria	RB41	Ellin6075
	6424250710a6c8f3ecd0fd26603bcbdb	0.52	0.04	3.9E-04	0	3.2E-04	0	0	0	Bacteria	Acidobacteria	Chloracidobacteria	RB41	Ellin6075
	959dc33fb13c310dd52835caa52eeca4	0.81	0.005	5.4E-04	6.7E-03	1.0E-02	5.4E-04	4.9E-05	0	Bacteria	Acidobacteria	Chloracidobacteria	RB41	
	4066eea496dc43011db89efa755d95ba	0.52	0.04	0	9.1E-04	1.5E-03	0	0	0	Bacteria	Acidobacteria	Chloracidobacteria	RB41	
	20b686910cf9db4b08f7b7c220800316	0.73	0.005	1.9E-03	7.3E-03	3.7E-03	1.0E-03	1.6E-03	0	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	
	83358a5ba1eb93b5fb9b0c26f03cfa16	0.63	0.005	2.2E-03	7.2E-04	3.4E-04	0	0	0	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	
	1c243c85d0ef1c7fb852db2ff20274c9	0.68	0.01	1.2E-03	1.3E-03	1.9E-03	0	0	0	Bacteria	Acidobacteria	Acidobacteria-6	iii1-15	

Table S2: *B. attenuatus* skin microbiota indicator species analysis results.

Assignment	ASV ID	LDA stats		*Eucalyptus* Sites			*Quercus* sites			Kingdom
		LDA	p.value	E1	E2	E3	N1	N2	N3	
Eucalyptus	df0534866a33108ad427b73b709ebfe8	0.63	0.005	1.2E-03	3.6E-04	5.5E-04	9.8E-05	7.8E-05	0	Bacteria
	e2d9476d14203066aa5c966011252216	0.62	0.015	9.8E-04	5.6E-05	1.0E-03	0	2.0E-05	0	Bacteria
	b379b1521d1a107507e12b3c376067b0	0.56	0.03	0	3.6E-04	1.0E-03	0	3.9E-05	0	Bacteria
	15d0c75db0d4705fd5dd4770a59c8f00	0.65	0.005	2.0E-04	7.0E-04	1.2E-03	0	0	0	Bacteria
	5f5dc2afebad265a4e4db64483f3f704	0.50	0.025	9.8E-05	1.4E-04	4.6E-04	0	0	0	Bacteria
	fcdee861c3a5b2efd76eb7d3ab1d606c	0.45	0.05	2.0E-04	5.9E-04	7.1E-05	0	3.9E-05	0	Bacteria
	20e7ada7880473eadf425735193501 47	0.57	0.005	3.2E-03	6.8E-03	5.0E-04	0	0	0	Bacteria
	477cc94cc17f6999ea63444cacaa470a	0.45	0.05	7.4E-04	4.2E-04	2.3E-04	0	7.8E-05	0	Bacteria
	3803258f892a9f8b6ee2e1b6de8436d4	0.53	0.025	1.1E-03	4.5E-04	2.5E-04	0	0	0	Bacteria
	7faccb65676a2c40636da004a18ec5a0	0.50	0.05	0	2.8E-04	1.1E-03	0	1.6E-04	0	Bacteria
	113b318d23356092ddae70f544df4718	0.46	0.035	7.8E-05	2.2E-04	3.6E-04	0	0	0	Bacteria
	6ced708f78dca8c0dd166df25149feb6	0.46	0.035	5.9E-05	2.5E-04	5.7E-04	0	0	0	Bacteria
	4479830df8a883aa2997024fadc88b52	0.46	0.045	5.9E-04	0	8.2E-04	0	0	0	Bacteria
	e007858c3abd7a15cf8e8930820a689e	0.53	0.015	2.9E-04	6.1E-04	2.8E-04	0	0	0	Bacteria
	5984c840c5cdeabb6540d4863e81270a	0.58	0.05	2.1E-03	8.9E-04	6.8E-04	4.9E-05	4.7E-04	0	Bacteria
	ad5d17028975c96a543bc1bdea4692f4	0.76	0.005	8.3E-02	2.1E-02	2.2E-02	0	1.2E-04	9.8E-05	Bacteria
	411512cc5d5ff7a557daeafffbfcc0dd	0.42	0.045	0.0E+00	5.6E-05	1.1E-04	0	0	0	Bacteria
	371675b527b024b935987d984e5e0adf	0.50	0.02	1.2E-04	1.4E-04	6.6E-04	0	0	0	Bacteria
	33fd454253b9038b04dc90dc9902d0dc	0.46	0.035	2.3E-04	3.4E-04	4.3E-04	0	0	0	Bacteria
	00e5c53e5de89d9e75ef2771ed62717f	0.55	0.015	8.0E-04	3.9E-04	2.8E-03	0	6.3E-04	0	Bacteria
	13d713ae52a5bf5fddfa3b92add63266	0.46	0.03	1.2E-04	1.4E-04	1.4E-04	0	0	0	Bacteria
	8f496bedd06ec30e808cda1970316225	0.42	0.045	1.6E-04	1.1E-04	2.1E-04	0	0	0	Bacteria
	a552c538964597136b53d5f7881f41e1	0.60	0.01	8.2E-04	1.1E-04	1.5E-03	0	0	0	Bacteria
	14331f18e634d3684c9185733a8c7be7	0.68	0.025	2.7E-04	2.0E-03	2.4E-03	2.0E-03	2.3E-04	7.3E-05	Bacteria
	980978a371cc5942be087bf324c5e2f3	0.57	0.005	7.2E-04	1.7E-04	3.0E-04	0	0	0	Bacteria
	822754e7f9add2d17f33255c21748e29	0.53	0.025	0	2.5E-04	2.0E-04	0	0	4.9E-05	Bacteria
	365ebfdf1a370ffbd233bf0dc8e22cab	0.63	0.01	2.9E-03	9.8E-04	5.7E-04	0	5.9E-05	3.2E-04	Bacteria
	26dced51f936f6ac6f6200bd49079161	0.71	0.005	6.5E-04	1.5E-03	6.3E-03	0	0	0	Bacteria
	42630c98d41bbf95b2a5040846 02f221	0.46	0.045	7.0E-04	1.4E-04	0	0	0	0	Bacteria
	2899612d084dff709dc7a2973a7121f6	0.46	0.03	4.7E-04	1.4E-04	1.4E-04	0	0	0	Bacteria
	999e7806b23a27b7759c33ac170411f7	0.64	0.005	3.9E-04	9.2E-04	1.7E-03	0	1.4E-04	0	Bacteria
	2511ff5d78ef6d53e9819a09ca5d9eea	0.53	0.01	5.9E-04	2.0E-04	3.9E-04	0	0	0	Bacteria
	a3b2563c2e0b9dc498975725b75fc560	0.50	0.035	2.3E-04	1.1E-03	5.9E-04	0	0	0	Bacteria
	c164fd002b5502c5963286a9322833a0	0.45	0.05	2.9E-04	9.8E-04	8.4E-04	0	0	1.5E-04	Bacteria

Take Home--What did they find?

1. No measurable changes in alpha soil microbial diversity, small portion due to beta community composition.
2. Community richness and phylogenetic diversity higher in *Quercus* habitat.
3. Body condition index higher in *Quercus* habitat.

Discussion Questions

1. Why was it surprising that only a small portion of microbial community structure could be explained by vegetation type?
2. What did the researchers mean by the skin microbiota having “host-level” effects?
3. What does the abundance of Chlamydiaceae ASVs on *Eucalyptus* salamander skin suggest?
4. What could be driving decline of body condition, if skin specific microbiota are not a factor?

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