EVALUATING MICROSHADE COLOR PALETTE ACCESSIBILITY FOR COLOR VISION DEFICIENCY

by

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TABLES, FIGURES, & ABBREVIATIONS

CVD - Color vision deficiency

OHSU - Oregon Health & Science University

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ABSTRACT

The field of microbiome research has grown exponentially over the past decade, marked by substantial investments in resources and recognition of its potential clinical significance. Microbiome data consist of highly dimensional and complex genetic information. Taxonomic abundance stacked bar plots are commonly employed in this field to offer a concise overview of the data, allowing researchers to identify specific taxa of interest for further analysis. These plots play an important role in simplifying data representation and facilitating hypothesis generation. Two of the most popular tools for microbiome data analysis are QIIME2 in Python and microbiome specific packages in R available through Bioconductor. Unfortunately, taxa bar plots using default colors in both QIIME2 and R are extremely difficult to read, particularly for those with color vision deficiency (CVD) who cannot perceive the entire spectrum of color and struggle to differentiate between certain colors. Although QIIME2 and R are both widely used analysis tools, they do not give users the option to apply alternate color palettes which are both CVD-accessible and include enough hues to guarantee that individual shades will be distinguishable. The *microshades* package, created by the Karstens Lab at OHSU, improves readability of taxonomic abundance stacked bar plots and provides a CVD-accessible color palette specifically designed for highly dimensional data. The CVD-friendly color palette developed for the *microshades* package offers a solution to current accessibility issues in highly dimensional scientific figures.

Our study aimed to answer two key questions. First, does the *microshades* color palette significantly improve accessibility of the plots published in microbiome literature for those with color vision deficiency? And second, how can we make CVD accessible color palettes widely available to microbiome researchers creating plots for publication? To answer the first question, we surveyed scientists with experience in either biology or microbiome science and asked participants to evaluate the accessibility of taxonomic abundance plots. These plots were shown in both their original published color palette and re-colored in the *microshades* CVD color palette. Surveys also showed figures simulated to appear as they would to someone with CVD. To answer the second question, we developed a QIIME2 plug-in which allows users to access the functionality and color palettes of the *microshades* package, providing farther-reaching visibility and access to a CVD-friendly taxa bar plot color palette throughout the microbiome research community.

Based on the results of our Microshades Accessibility Survey and subsequent analysis, we acknowledge the improvement in plot accessibility and interpretation accuracy achieved through the use of the *microshades* CVD color palette. However, we also recognize that the impact was less pronounced for most CVD participants, possibly due to the lower-than-expected effect size and limited number of participants diminishing the statistical power. Overall, our findings emphasize the importance of accessible visualizations in scientific research and warrant further investigation into the practical implementation of the *microshades* CVD color palette in accessibility studies.

INTRODUCTION

Human Microbiome Research

Microbiome research has grown exponentially over the past decade. The microbiome has been connected to several diseases, including multiple sclerosis[1], inflammatory bowel disease[2], diabetes[3], Parkinson's disease[4], colorectal cancer[5], Alzheimer's disease[6], and depression[7] to name a few. It is estimated that the number of publications pertaining to the human gastrointestinal microbiome increased from just 67 articles published in the year 2010 to over 1,100 in published in 2020.[8]

The human body plays host to entire ecosystems of living microorganisms, collectively termed "microbiota." These organisms include bacteria, viruses, archaea, fungi, and eukaryotes living in a delicate equilibrium within and on the surface of our bodies, and they outnumber our native somatic and germ cells ten-to-one.[9] In the past, the microbiota could only be studied using traditional culture-based methods. However, new methods use DNA sequencing technology to sequence the "microbiome", genetic information contained within microbes that make up our microbiota. [9] These methods allow for the accurate and rapid taxonomic classification of microorganisms within a sample. Microbial taxonomy is the organizational hierarchy by which micro-organisms are named and grouped together. **Figure 1** gives an example with the *Lactobacillus helveticus* species.

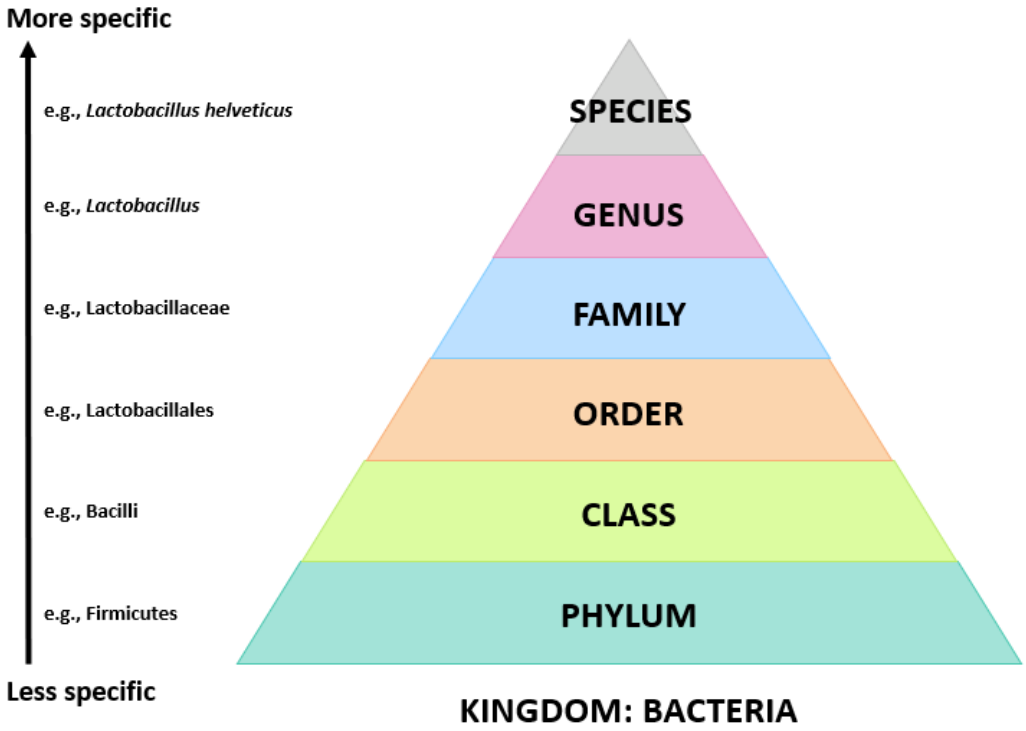

Figure 1. *Taxonomic ranking of microbiota[10]*

Microbiome data consist of highly dimensional and complex genetic information from all microbes in a sample - often hundreds of species.[11] The sequencing information is grouped into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs), based on a set threshold of divergence. Taxonomic ranking (e.g., phylum, family, genus, species) is assigned to each OTU/ASV by mapping to reference databases.[12] Samples are compared by assessing microbial diversity measurements such as the presence, absence, and abundance of taxa.[12] However, due to the vast number of taxa present in any given sample, microbiome studies struggle with an inherent lack of statistical power.[12] To ameliorate the issue of high dimensionality, data visualizations are used to "visualize potential clustering by clinical variables in an unsupervised way," so that identified clusters can be statistically evaluated to determine if there is biological significance.[12]

Data Visualizations

One commonly used visualization in a microbiome researcher's arsenal is the taxonomic abundance stacked bar plot, or taxa bar plot. There are two types of taxa bar plots - absolute and relative. Taxonomic absolute abundance plots show absolute quantities of each taxon, while taxonomic relative abundance plots show taxa quantities as a percentage of the sample's total taxa. The taxa bar plot is a visual representation of all taxa identified within each sample and their abundance arranged alongside one another for side-by-side comparison, as shown in **Figure 2**. Visualizing this complex information allows analysts to pinpoint specific taxa of interest for further analysis.[12] Taxa bar plots are also used for exploratory data analysis and quality control. Each sample loci (tongue, feces, gut, etc.) is characterized by a handful of signature taxa that are frequently identified that environment.[12] For example, the vaginal microbiome is commonly characterized by *Lactobacillus*, a genus of fermentation bacterium.[13] Visualizing microbiome data in a taxa bar plot may shed light on any data quality issue requiring further investigation.

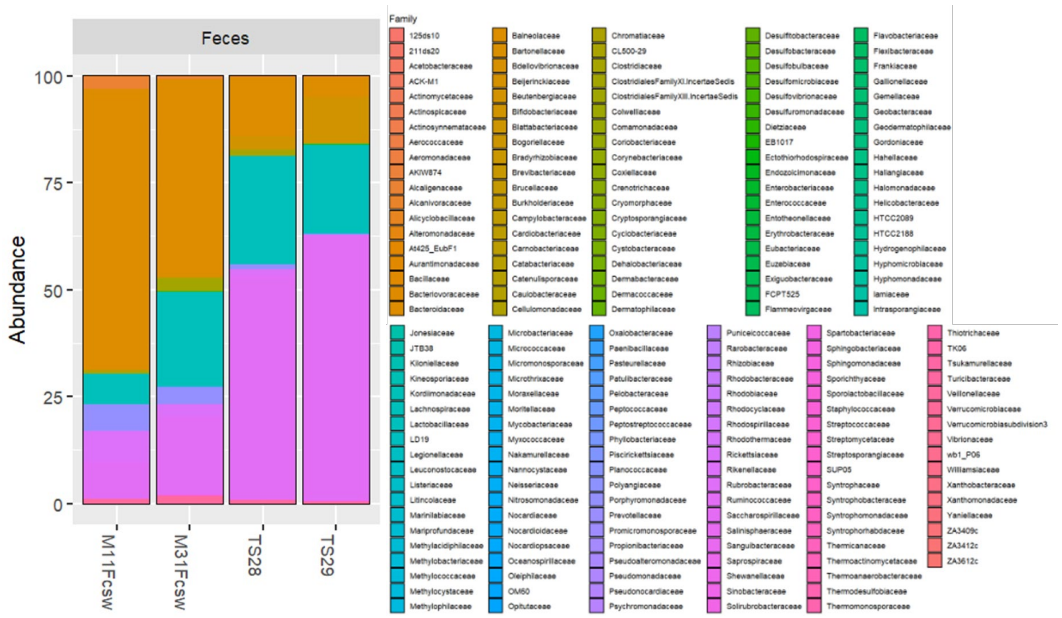

Figure 2. *Example taxonomic relative abundance stacked bar plot generated in R using phyloseq*[14]

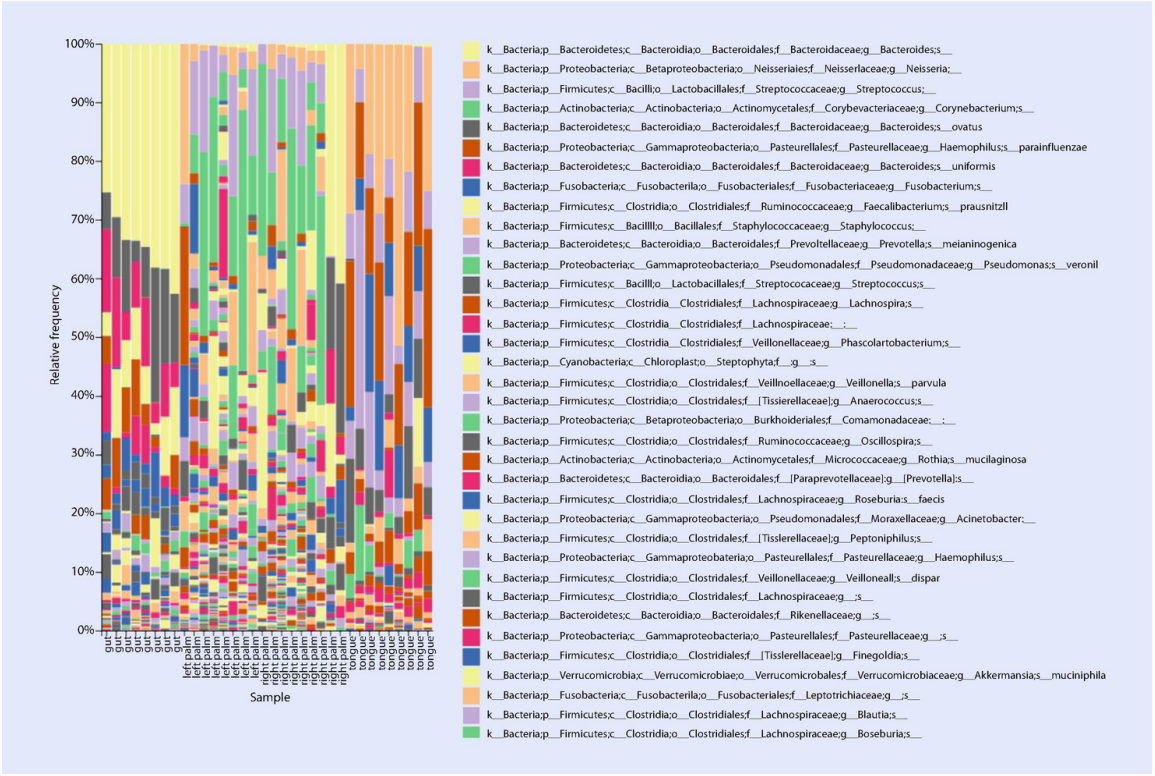

Figure 3. *Example taxonomic relative abundance stacked bar plot generated in Python using QIIME2*[15]

Two of the most popular tools for microbiome data analysis are Bioconductor in R and QIIME2 in Python.[12] Both are powerful tools which provide pipelines to take raw DNA sequence data and create publication-quality figures and statistical results. However, there are a few notable differences between the two tools. Bioconductor is a compilation of many packages with broad applications to handle any genetic data analysis.[14] Partially because of its broad application, Bioconductor requires a higher level of familiarity with coding and is considered less friendly to novice programmers.[12] QIIME2, on the other hand, is a tool specifically developed for microbiome data analysis.[15] Its streamlined workflows, ease of use, and volume of high-quality community documentation and support make it extremely popular with scientists.[15] Both Bioconductor and QIIME2 are open-source and allow for community collaboration.

Unfortunately, taxa bar plots using default colors in both Bioconductor and QIIME2 are extremely difficult to read.[16] **Figure 2** above was created using the phyloseq package within Bioconductor and uses the package's default color palette. The plot attempts to use a continuous color palette to represent highly dimensional, categorical data. In the legend, we see that many of the individual colors assigned to different taxa families are indistinguishable from one another. Of the less than ten colors identifiable to the naked eye in this plot, it is effectively impossible to tell which family they are meant to represent based on color alone. QIIME2 suffers from a similar issue. **Figure 3** above was created using QIIME2 and also uses the package's default color palette which is not extensive enough for microbiome datasets. The limited number of colors are repeated to represent all taxa groups, and the repeated colors are not all easily interpretable. Readability issues are exacerbated for those with CVD who cannot perceive the entire spectrum of color and struggle to differentiate certain colors from one another.[16] Although tools such as Bioconductor and QIIME2 are widely used, they do not give users the option to apply alternate color palettes which are both CVD-accessible and include enough hues to guaranteeing individual shades will be distinguishable when visualizing highly dimensional data.[16]

The microshades R Package

The *microshades* package was created by the Karstens Lab at OHSU to improve the readability of taxonomic abundance stacked bar plots, an essential tool in microbiome data visualization and analysis.[16] This package allows users to organize data by taxonomic ranking and provides two color palette options, one of which is specifically designed to be CVD-accessible as shown in **Figure 4** below. Each color palette has 30 unique, clearly distinguishable colors. *microshades* was initially developed to enhance the visualization of microbiome data stored in phyloseq objects in R; however, the package can be used for any type of plot.[16]

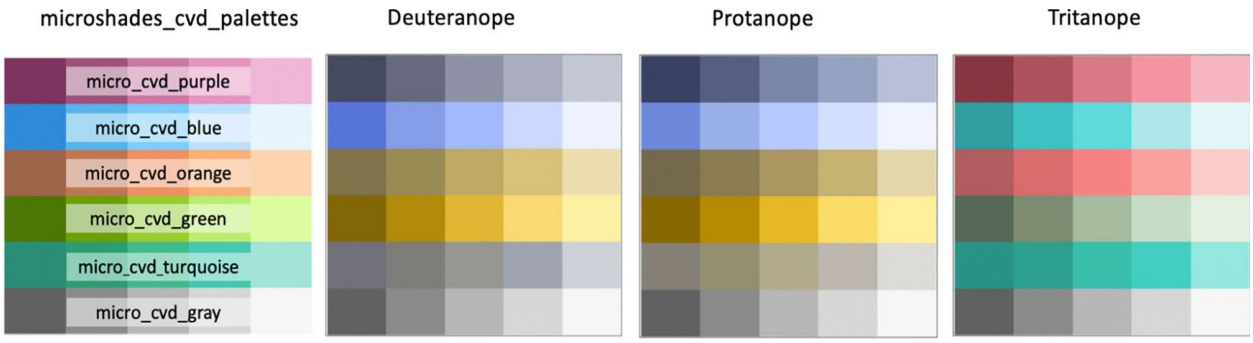

Figure 4. *The microshades CVD-accessible color palette simulated in deuteranope, protanope, tritanope*[16]

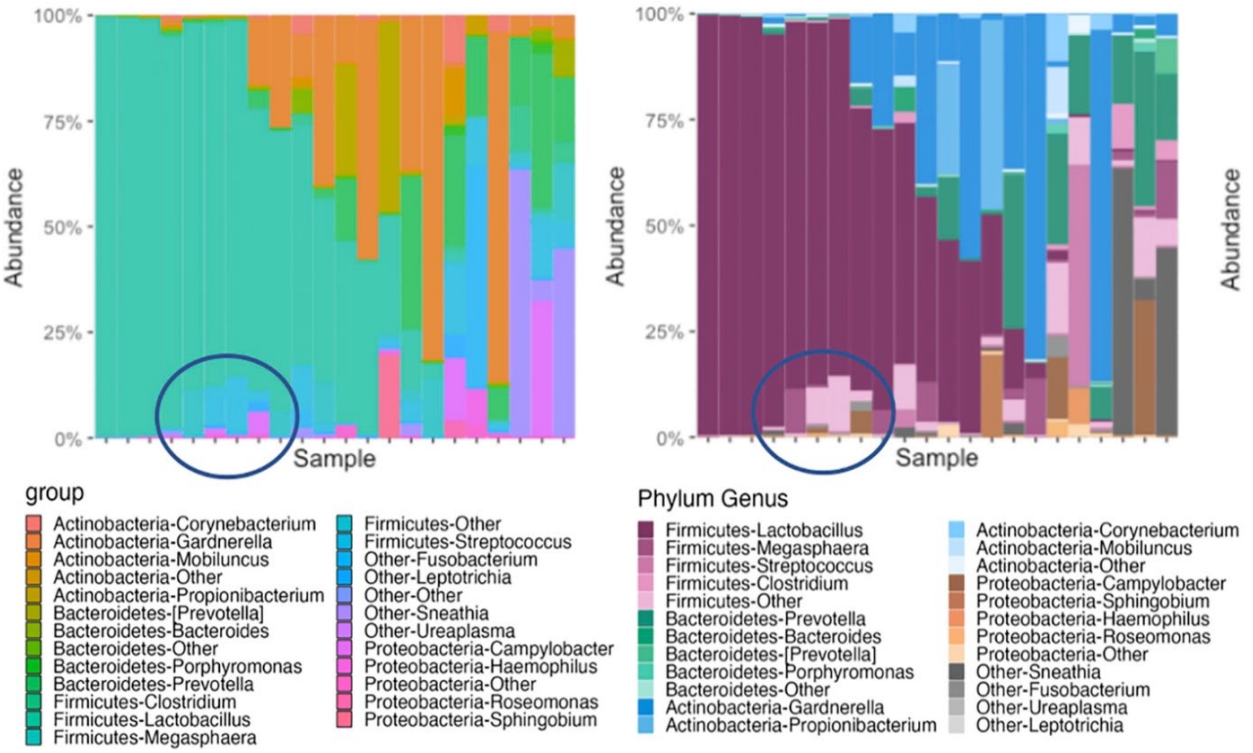

Figure 5. *Example taxa bar plot comparing default color palette in R (left) versus the microshades color palette (right)*[16]

Figure 5 shows two versions of the same taxa bar plot, one created with a default color palette in R (left) and the other created with the *microshades* CVD color palette (right).[16] Circled in each plot is a particularly difficult section of the plot to read in default colors which is then resolved using the *microshades* color palette. The CVD-friendly color

palette developed for the *microshades* package offers a solution to current accessibility issues in highly dimensional scientific figures.

Color Vision Deficiency

The human retina has three types of color-sensitive photoreceptors, or "cones". Each type of cone absorbs a certain spectrum of light: short wavelengths, medium wavelengths, and long wavelengths.[17] These are often called "blue", "green", and "red" cones, respectively, according to the spectra of light they process.[17] The human brain perceives color by comparing activity between these three types of cones.[17]

Color vision deficiency (CVD), commonly referred to as "color blindness", is a condition affecting cones within the retina caused by either a congenital genetic mutation or an acquired injury.[17] For those with CVD, the perceived spectrum of light for one type of cone is shifted or one type of cone is missing entirely.[17] Anomalous trichromacy - when one type of cone's perceived light spectrum is shifted - causes the color spectra of two types of cones to overlap, making it extremely difficult if not impossible to distinguish between the two colors.[17] Dichromacy - when one type of cone is missing - prevents the perception of a sub-spectrum of light altogether.[17] Monochromacy - when more than one type of cone is missing - means that color cannot be perceived at all.[17] Color vision deficiencies are divided into three categories: deuteranope ("red"-cone mutation), protanope ("green"-cone mutation), and tritanope ("blue"-cone mutation).[17] Both deuteranope and protanope cause difficulty differentiating between red and green, hence the commonly used term "red-green color blindness", while tritanope, the rarer of the three, causes difficulty differentiating between yellow and blue.[17]

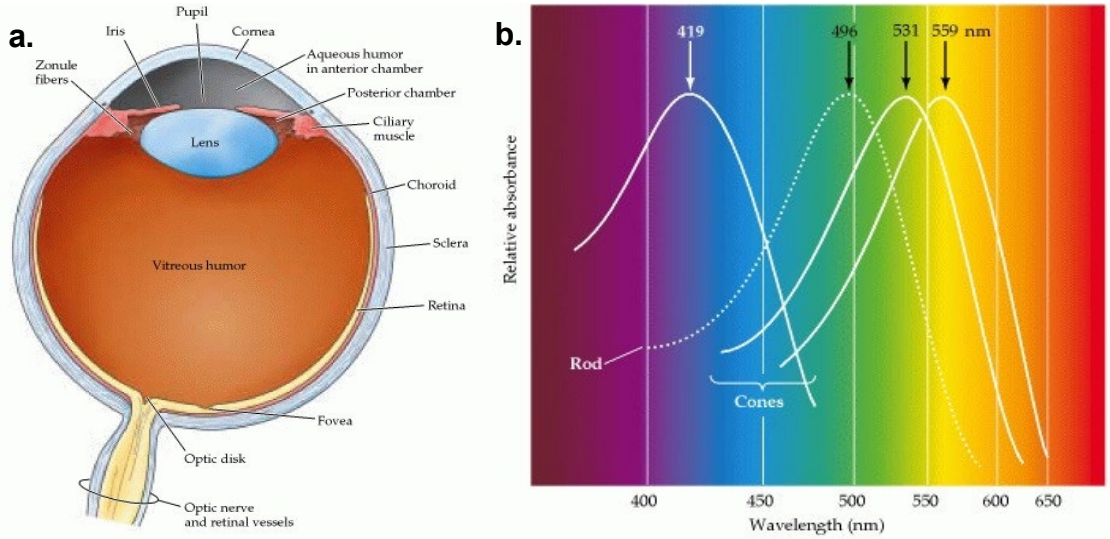

Figure 6. *(a) Anatomy of the human eye and (b) spectra of light for each type of cone*[17]

Worldwide, CVD affects approximately 8% of men and 0.5% of women.[18] This means that a group of people roughly equivalent to the population of the United States cannot perceive the entire color spectrum and may struggle to interpret color-coded information.[18,19] Abnormal color vision can have detrimental impacts throughout an individual's life, complicating daily routine tasks, education, and occupation.[20]

For those with CVD, scientific figures are particularly difficult to interpret.[21,22,23] Scientific figures rely on color to convey information; however, few scientists are formally trained in data visualization and color design.[22] Reports show that figures are the first element readers examine when reading a paper.[24] Color selection is a critical design consideration when creating figures to convey findings and concepts to any audience. For example, a presentation at a conference of 1,000 people will likely have at least 40 audience members with a color vision deficiency. And during the peer-review process for publication, there is a 20% chance that one of the paper's reviewers will have some type of CVD if all three are male and have northern European ancestors.[25] Given its prevalence in the global population, authors should consider CVD accessibility when creating scientific figures.

Accessibility guidelines exist for images and text in web pages and web applications.[26,27] Current accessibility standards include The Web Content Accessibility Guidelines 2.2 (WCAG) and US Federal Guideline Section 508, which refers to compliance level AA of the WCAG.[27] The WCAG were created to make online content more accessible to a wide range of people with disabilities, including "blindness and low vision, deafness and hearing loss, limited movement, speech disabilities, photosensitivity, and combinations of these,".[26] These guidelines include three distinct levels of compliance (A, AA, and AAA) specifying recommendations such as the required contrast ratio between adjacent colors, required text size, and labeling requirements to improve readability.[26] Level AA, for example, requires a 3:1 contrast ratio for adjacent colors (Section 1.4.11).[26] Platforms for accessibility testing allow developers to automatically review a website or image according to WCAG and ADA accessibility requirements.[28] Color blindness simulators are another recommended tool for evaluating and improving accessibility as they allow developers to view a website or image through the eyes of those with color vision deficiency and identify possible accessibility concerns.[29]

Guidelines such as the WCAG and CVD simulation tools represent substantial steps in the direction of accessibility. However, these broad guidelines are not sufficient for the visual complexity of scientific figures. In recent years, several studies have investigated the accessibility of published scientific figures, in particular for those with low vision and CVD.[21,22,23]

In 2015, a study by Frane et al examined the CVD accessibility of 246 figures from psychology published literature.[21] The study assessed figures in three stages. First, the author (full color vision) used a CVD simulator in Photoshop to simulate a dichromatic

view before examining all 246 figures. Frane flagged 38 figures as “confusing” where important information was lost in the simulation and the loss of information could be resolved by simple changes in color. Second, a panel of 5 volunteers with CVD reviewed the 38 flagged figures to compare the originals versus the same figure with color corrections. In this stage, the 5 volunteers confirmed that 20 of the original figures were confusing. Finally, a panel of 10 volunteers with full color vision reviewed the 20 confusing figures to compare the two versions of each figure - original and color corrected. The final panel confirmed that the changes in color did not negatively affect the readers’ ability to interpret the figures. Frane also reviewed the publication requirements of psychology journals in search of recommended CVD accommodations for figures. The study by Frane et al presented two important findings: (1) 8% of figures reviewed were not CVD accessible and (2) less than 1% of psychology journals recommend CVD accommodations for figures.[21]

In a 2021 study by Jambor et al, two researchers systematically reviewed visual accessibility of image-based figures from 580 papers in the fields of cell biology, plant sciences, and physiology.[22] The figures reviewed included microscope images, photographs, electron microscope images, and clinical images. The reviewers examined figures for key accessibility factors such as “the use of scale bars, explanations of symbols and labels, clear and accurate inset markings, and transparent reporting of the object or species and tissue shown in the figure”.[22] The reviewers also simulated figures with deuteranopia, the most common form of CVD, with Color Oracle, a CVD simulator, to assess CVD accessibility.[22] The study by Jambor et al found that 45% of cell biology papers had at least one figure inaccessible to those with deuteranopia.[22]

A recent study by Angerbauer et al published in 2022 expanded on the work of both Frane et al and Jambor et al.[23] The study examined the CVD accessibility of 1500 scientific figures from the IEEE image database in two stages. First, four of the study authors examined a subset of 210 figures to identify themes of helpful and problematic accessibility factors across images. Second, the authors surveyed a group of 200 crowdsourced Amazon workers to examine all 1500 figures. Workers were asked to compare a trichromatic (full color) figure and the same figure simulated with either anomalous trichromacy or monochromacy. The workers were prompted to list helpful and problematic accessibility factors for each figure according to themes identified in the first image review stage and give an accessibility rating based on the simulated version of the figure. The study by Angerbauer et al presented two important findings: (1) classification of key accessibility factors, such as resolution, labels, and figure complexity and (2) 8% of figures reviewed were not CVD accessible.[23]

These three studies clearly establish the prevalence of accessibility issues in scientific images and figures. Although all three provide extensive recommendations to improve figure accessibility, it is also clear that journals are either not translating suggested guidelines into publication requirements or not enforcing their figure accessibility requirements. To our knowledge, there has yet to be a study investigating the

accessibility of more complex quantitative figures, such as the taxa bar plot, or reviewing a comprehensive CVD-accessible color palette with a sufficient number of colors for highly dimensional, categorical data.

MATERIAL & METHODS

Specific Aims

Our study aimed to answer two primary questions. First, does the *microshades* color palette improve accessibility of the plots published in microbiome literature for those with CVD? And second, how can we make CVD accessible color palettes widely available to microbiome researchers creating plots for publication? In order to answer these two questions, we defined the following specific aims.

Specific Aim #1 - Evaluate the accessibility of *microshades* CVD color palette in taxonomic abundance bar plots for those with color vision deficiency.

To accomplish this aim, we surveyed scientists with experience in the biology or microbiome fields. For the survey, we:

- Selected 8 taxonomic relative abundance stacked bar plots from publications according to a defined set of requirements.
- Resized and recolored the plots in the *microshades* CVD color palette.
- Transformed all plots (both original and recolored) using the Coblis Color Blindness Simulator.[29]
- Surveyed scientists to perform simple interpretations of all plots, assess their confidence levels, and evaluate plot accessibility.
- Analyzed survey results to quantify the improvement in accessibility of the *microshades* CVD color palette as compared to color palettes currently used in published work.

Specific Aim #2 - Prepare the *microshades* CVD color palette as a QIIME2 plug-in.

To accomplish this aim, we:

- Defined system requirements for the plug-in.
- Wrote a Python wrapper to run functions from the *microshades* R package in a background instance of R.
- Wrote a plug-in set up script to register all *microshades* functions per the QIIME2 plug-in development requirements.

- Tested all Python wrapped functions with sample microbiome data from a QIIME2 vignette.
- Created the necessary documentation files.
- Submitted a pull request to add the plug-in to the q2-microshades repository in the KarstensLab GitHub.
- Time permitting, we will request admin approval from QIIME2 for the new plug-in and publish the package to the QIIME2 Library as an official plug-in.

Study Design

Study data were collected and managed using REDCap electronic data capture tools hosted at OHSU.[30,31,32] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources. The survey was a self-administered questionnaire written in English and accessed via a public link. The survey consisted of two sections: 1) general demographic information and 2) taxa bar plot evaluation. In the demographic section, participants were asked to self-report their biological sex assigned at birth, highest education level, experience, and any CVD diagnosis or other visual impairments. In the evaluation section, each question presented one taxa bar plot, either in the published plot's original colors or recolored in the *microshades* CVD color palette.

To select relevant figures for this survey, we performed a systematic review of figures published in microbiome literature according to a defined set of requirements. To be included in this study, figures needed to:

1. Be a taxonomic relative abundance stacked bar plot
2. Have been published during or after the year 2020
3. Have x- and y-axes present
4. Have an ordered legend present
5. Have taxa only distinguished by color (no outlines or texture differentiation)

In addition to these requirements, figures were also selected for similar image resolution. These requirements helped to ensure color was the only independent variable for accessibility and readability within the survey.

Image processing was automated in R using the *imager* R package, a tool for advanced image processing and manipulation.[33] All images were first cropped down to a single bar in the stacked bar plot. The cropped images were standardized to the same height and width, and uniform, ordered legends with representative taxa names (i.e. Bacteria A, Bacteria B, etc.) were created for each cropped plot. These resized images were then automatically recolored in R. The plot's original colors were each identified in terms of RGB values, the original colors were replaced with colors corresponding to the *microshades* CVD color palette, and a new version of the image was generated. The plot in both its original published colors and recolored were used in the survey as separate questions. Documenting the image manipulation process as an automated process may help to facilitate any future replication of our study.

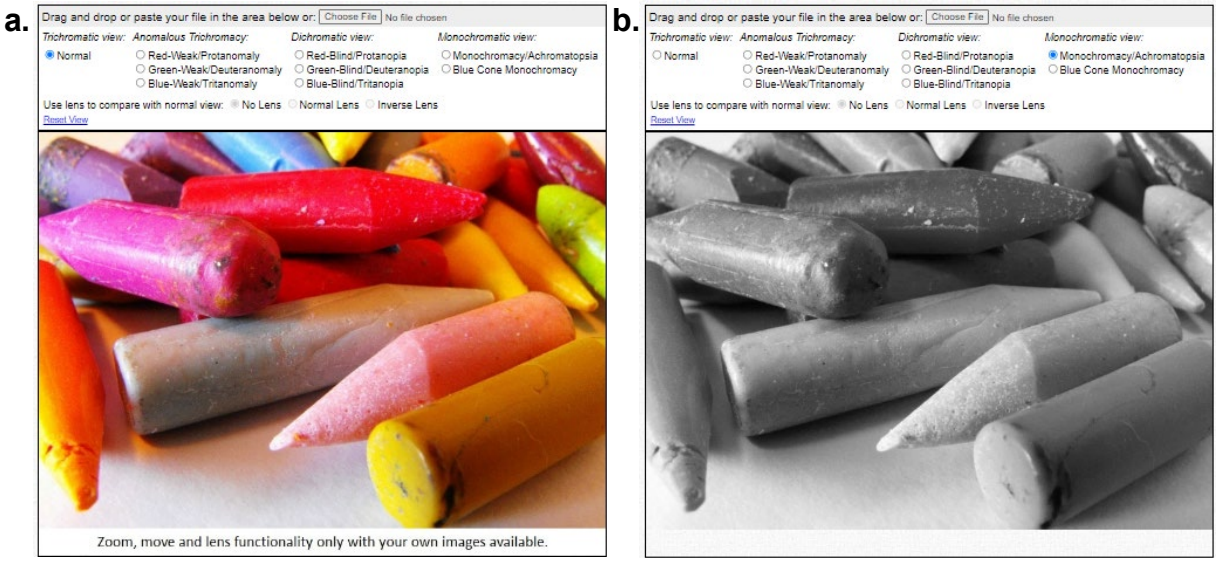

Figure 7. *Coblis Color Blindness Simulator web application showing an image of colorful crayons in the (a) Normal Trichromatic view and the (b) Monochromacy/ Achromatopsia simulation view*[29]

Both original and microshades versions of the taxa bar plots were also simulated to show the plots' colors as they would appear to someone with CVD. To simulate CVD, we used the Coblis color blindness simulator[29], a commonly used CVD simulator.[34] **Figure 7** above shows the Coblis web application in a Chrome browser with the default image of colorful crayons. This simulator takes uploaded images and transforms the images' colors to represent the effects of either an anomalous trichromatic, dichromatic, or monochromatic view. The image on the left (a) shows the uploaded image without any simulations, and the image on the right (b) shows the same image with the Monochromacy/Achromatopsia simulation view. Coblis employs the HCIRN Color Blind Simulation algorithm by Matthew Wickline and the Human-Computer Interaction

Resource Network to transform the image. This algorithm treats the image as a matrix of RBG values and mathematically manipulates that matrix.[35,36] Simulating CVD for the simulation subgroups allowed us to treat participants with full color vision as participants representing the CVD population in our analysis. When simulating images for our surveys, we used the following simulation views: Red-Blind/Protanopia, Green-Blind/Deuteranopia, Blue-Blind/Tritanopia, and Monochromacy/Achromatopsia. We did not select any of the lens options in these simulations.

For each plot in the evaluation section, participants were asked to (a) interpret a piece of information in the plot, (b) report their level of confidence in that interpretation, (c) rate the plot's accessibility, and (d) explain their accessibility rating in a free-form textbox (see Appendix). The interpretation question encouraged participants to pause, interact with, and critically evaluate the plot before rating its accessibility. The accessibility rating was our primary focus for analysis, and it allowed us to compare our results with those of prior accessibility studies. Participants were all given a definition of each accessibility rating – "accessible", "borderline", and "not accessible" – before evaluating any plots to ensure consistency.

Each of the 10 taxa bar plots in the survey were shown twice – once in the plot's original color palette and once recolored with the *microshades* color palette – with the same interpretation question posed for both versions. Together, this resulted in an Evaluation Section with 16 questions in total. Interpretation questions specifically targeted taxa which were deemed difficult to read in the CVD simulations with the plots' original color palettes based on the consensus of two investigators familiar with taxa bar plots. Questions evaluating original and re-colored plots were evenly distributed from the beginning to the end of the survey, and the original plot and its recolored counterpart were never in direct sequence in the survey (see Appendix).

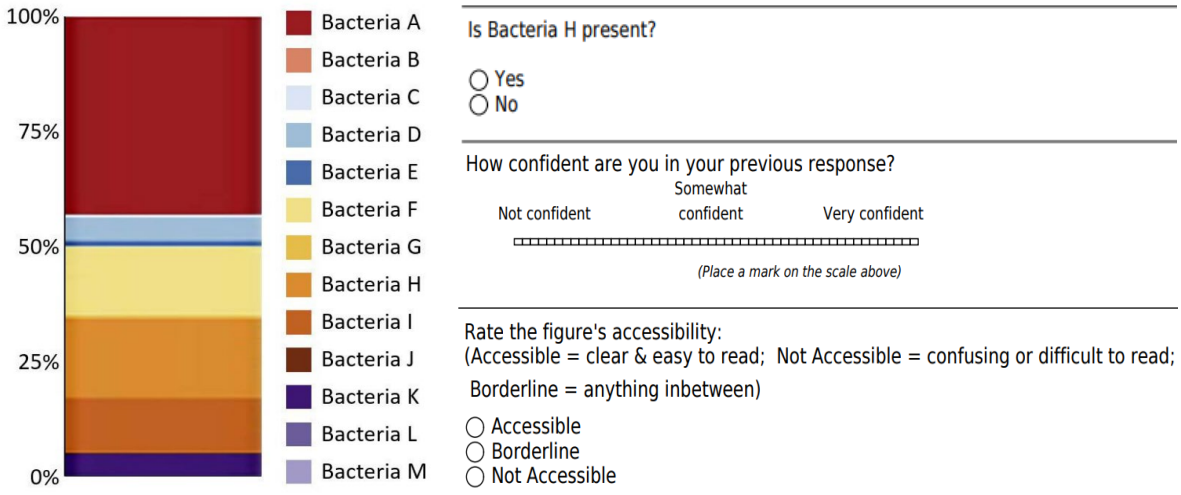

Figure 8. *A survey question from the CVD version of the Microshades Accessibility Survey*

Our study consisted of 3 cohorts: a pilot group, a CVD cohort, and a Full Color Vision cohort. Five full color vision volunteers made up our Pilot group, and the purpose of this group was to verify the survey time, the questions, and the overall user experience for the survey before launch. The CVD cohort included 10 participants who were diagnosed with CVD, and these participants took the survey without any simulations. The purpose of the CVD cohort was to verify the results of the simulation groups with accessibility ratings and plot interpretations from CVD participants. It is crucial to include those who have been diagnosed with a color vision deficiency in any CVD accessibility study because, although they are extremely useful tools[37], simulations do not perfectly represent the experience of someone with vision deficiency.[38] The full color vision cohort included 100 participants with full color vision who were assigned to one of 5 subgroups. The purpose of the Full Color Vision cohort was to evaluate the accessibility of the *microshades* CVD color palette in taxonomic abundance bar plots for those with color vision deficiency by simulating CVD vision for participants with full color vision. These subgroups were either given surveys without CVD simulation for the control group or surveys with figures simulated to represent the type of CVD corresponding to each of the subgroups. We opened the survey to participants with CVD and full color vision simultaneously.

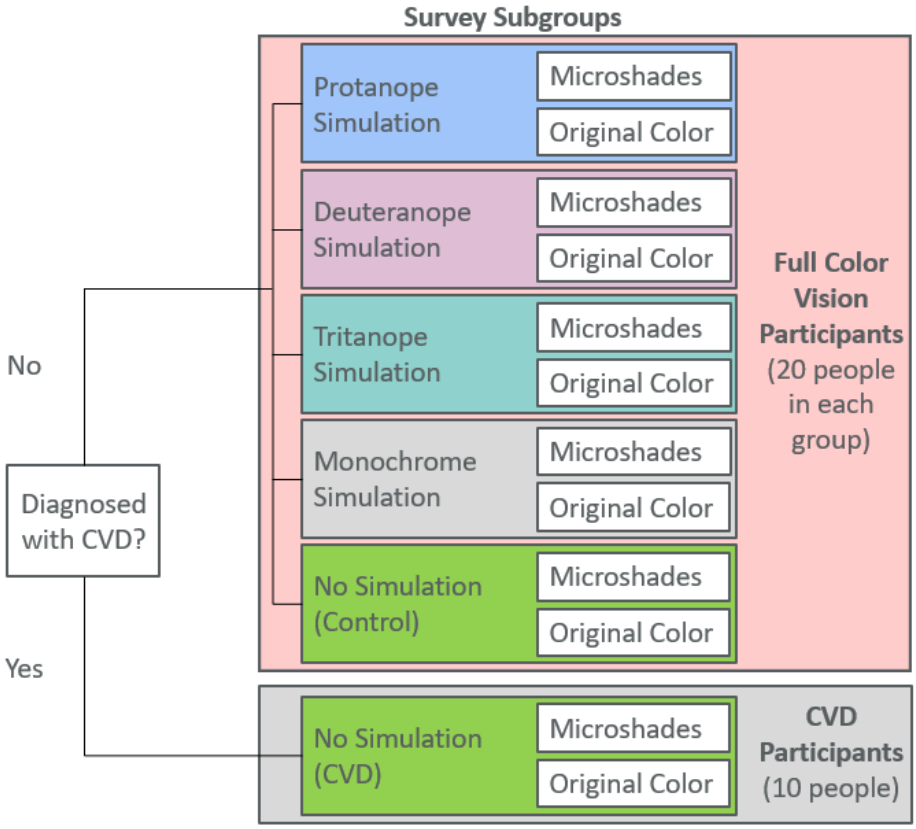

Figure 9. *Survey's built-in logic assigning participants to survey subgroups*

The five Full Color Vision survey subgroups each completed a different version of our survey. All five versions of the survey had the same structure, plots, and questions, but four of the survey versions had taxa bar plots simulated to represent one of four different types of color vision deficiency: Deuteranopia, Protanopia, Tritanopia, and Achromatopsia (Monochromacy). **Figure 9** above shows all five survey versions, the colors of taxa bar plots included in each version (original and *microshades*), and the logic built into our surveys to determine which participants will be assigned to which survey version.

Subjects

Our study consisted of 3 cohorts: a pilot group, a CVD cohort, and a Full Color Vision cohort. Before officially moving the survey to production, 5 volunteers with full color vision completed an initial test version of the survey via OHSU-approved REDCap. After this, the survey was open to participants with and without CVD at the same time. Participants were automatically assigned to a group based on their response to the survey's demographic question about CVD diagnosis. In the CVD phase, more than 5

participants with CVD completed the survey via REDCap. In the Full Color Vision cohort, 100 participants without any CVD diagnoses (assumed to have full color vision) completed one of five versions of our survey via REDCap. Each of the 100 full color vision participants were randomly assigned to one of five survey subgroups: four CVD simulation groups and one control group. The four groups taking CVD simulated surveys – Deuteranopia, Protanopia, Tritanopia, and Monochromacy – acted as "CVD representatives" in our study.

Surveys are conducted to estimate the response of a larger population by querying a small subset of that population. In order to extrapolate survey results from our sample size to the larger population, we calculated a rough estimate of the number of biologists with a color vision deficiency using two pieces of information: the US Bureau of Labor National Estimates there are 139,890 Biological Scientists (Soil & Plant Scientists, Biochemists and Biophysicists, Microbiologists, Zoologists and Wildlife Biologists, All Other Biological Scientists, All Other Life Scientists)[39] working in the US and the prevalence of CVD in the US population is estimated to be around 3.7%.[40] With this information, we then estimated the number of survey participants required for the final simulation phase using the equation below:

$$N = \frac{\frac{p(1-p) * Z^2}{e^2}}{1 + \frac{p(1-p) * Z^2}{ne^2}}$$

where N = sample size, n = population size, p = population proportion, e = margin of error, and z = Z-score.[41] The Z-score is a factor of the desired confidence interval (CI), which is a percentage describing the likelihood that a result lies within the margin of error.[41] The margin of error is a percentage describing the chance that results will not reflect the true response of the larger population. Both the margin of error and the confidence interval (and the Z-score, by extension) are selected. Standard values used in survey research for the margin of error and confidence interval are 1%, 3%, or 10% (MOE) and 90%, 95%, or 99% (CI), respectively. The results of all calculations mentioned above can be found in **Table 1** below.

Table 1. *Estimating survey participants required for the four CVD groups in the simulation phase of the study*[39]

Description	Value
US Bureau of Labor National Estimates for Biological Scientists (2021)	139,890
Percent of US Population with CVD	3.7%

Estimate of Biological Scientists with CVD in the US	5,176
Ideal Sample Size (CI 95%, MOE 5%)	358
Useful Sample Size (CI 90%, MOE 10%)	68
Our Target Sample Size (Simulation groups- deuteranope, protanope, tritanope, and monochrome groups combined)	80

Survey participants were recruited via four primary methods: conference announcements, posted fliers, tweets, and direct email. Dr. Karstens and other members of the Karstens Lab recruited participants at the following 2023 conferences: the Lake Arrowhead Microbial Genomics Conference, the Center for Microbiome Innovation Urobiome Conference, and the Microbiome Virtual International Forum Conference. Fliers were posted at the OHSU campus around the library. Dr. Karstens posted a total of two tweets (messages posted on the Twitter online messaging service) promoting the survey and asking for interested participants. These were reposted several times, increasing the reach of our recruitment method. Administrators of the Department of Medical Informatics and Clinical Epidemiology at OHSU sent a department-wide email with information about the survey inviting all to participate. The promotional material contained either a short URL or a QR code giving participants access to the survey. Participants were also recruited at the OHSU Research Week Poster Presentations. All promotional material can be found in the Appendix.

Data Collection

All data were collected and stored in REDCap, an OHSU approved secure web application for creating and managing online surveys and databases in compliance with HIPAA and IRB requirements.[32] REDCap is protected by OHSU firewalls and backed up to OHSU servers. All methods of data collection were reviewed and approved by the REDCap Team before launch. Surveys collected necessary participant health information such as vision impairment and CVD diagnoses; however, identifying personal information such as names, ages, or email addresses were **not** collected.[42] All surveys were completed anonymously to ensure confidentiality, and REDCap automatically assigned record IDs to each survey as they are completed. Our survey questions, data collection, and recruitment methods were approved by IRB before launching the survey.

Data Analysis

Our analysis primarily focused on understanding the difference in accessibility ratings between two color palette categories - the original color palette (i.e., "original") and the *microshades* CVD color palette (i.e., "microshades"). For each survey, we analyzed the average percent of figures rated "accessible", "borderline", and "not accessible" for both the original color palette and *microshades* color palette by survey group. The average percentage was determined by separating the results into two sets: those involving microshades and those with original plots. For each participant and each color palette, the percent of "accessible", "borderline", and "not accessible" ratings were calculated. These individual participant percentages were then averaged across all participants in each survey subgroup.

To compare the average percentages between the microshades and original plots for each survey subgroup, we also calculated the difference in the average percent of figures rated as "not accessible." We specifically focused on the "not accessible" ratings in our analysis to understand the effects of the *microshades* CVD color palette on the most problematic plots.

In addition, we analyzed the accessibility ratings by question. We separated the results by survey subgroup and found the percent of participants who gave each accessibility rating ("accessible", "borderline", and "not accessible") for a given taxa bar plot in either the microshades or original color palette. As with the average group percentages, we calculated the difference between the percent of participants rating the plots as "not accessible" with the microshades color palette versus the original color palette for each survey question.

We employed McNemar's exact chi-squared test to analyze the accessibility rating results for each survey subgroup.[43] This test is a statistical method designed for paired binary response data, for example cases where twins are randomized into two treatment groups and tested for a binary outcome (i.e., pass or fail). In the case of our analysis, the pairs are the two versions of the same taxa bar plot – one with the original color palette and the other with the *microshades* CVD color palette – both individually assessed by the same participant. This approach considers four possible outcomes for each pair, classified as (a) both plot versions are rated as "not accessible", (b) the control version is rated "not accessible" while the microshades version is rated either "accessible" or "borderline", (c) the microshades version is rated "not accessible" while the control version is rated either "accessible" or "borderline", or (d) both versions are rated as either "accessible" or "borderline" (**Table 2**). To assess the effectiveness of the *microshades* CVD color palette, this test focuses on discordant pairs (b and c). The exact version of the McNemar's test is useful when inferences are sensitive to changes and can be applied to a smaller sample size, requiring a minimum of 10 discordant pairs for any group evaluated.[43]

Table 2. *Contingency table for accessibility ratings in a given survey subgroup*

	Accessible/ Borderline	**Not Accessible**
Accessible/ Borderline	*a*	*b*
Not Accessible	*c*	*d*

Our null hypothesis for this test was that the marginal probabilities for the two categories – "accessible/borderline" and "not accessible" – were the same for both the *microshades* and the original color palette. Our alternate hypothesis was that the marginal probabilities for the two categories were not the same and depended on the color palette used in the plot.

We used a similar analysis method for the surveys' interpretation results: first, calculating the average percent of questions answered incorrectly for both the original palette and *microshades* palette across each survey subgroup and then calculating the difference in average percent of incorrect interpretations between the original and microshades plots. We also found the percent of participants who answered the interpretation question incorrectly for a given taxa bar plot in either the microshades or original color palette and the percent difference between those two results. Again, McNemar's exact chi-squared test was employed to analyze the interpretation accuracy results for each survey subgroup (**Table 3**).

Table 3. *Contingency table for interpretation accuracy in a given survey subgroup*

	Correct	**Incorrect**
Correct	*a*	*b*
Incorrect	*c*	*d*

Our null hypothesis for this test was that the marginal probabilities for the two categories – "correct" and "incorrect" – were the same for both the *microshades* and the original color palette. Our alternate hypothesis was that the marginal probabilities for the two categories were not the same and depended on the color palette used in the plot. Analysis of the interpretation results serves as important supporting evidence for the primary accessibility results.

In the survey, participants reported their confidence on a scale of 0-10. This scale was consolidated into three discrete levels – "high", "medium", and "low" confidence. The boundaries of each level were established by analyzing the distribution of confidence ratings across all participants and identifying local minima within the distribution. Again, we used a similar analysis method for the surveys' confidence results: first, calculating the average percent of questions answered with "high", "medium", and "low" confidence levels for both the original palette and *microshades* palette across each survey subgroup and then calculating the difference in average percent of "low" confidence levels between the original and microshades plots. We also found the percent of participants whose rating fell into each confidence level for a given taxa bar plot in either the microshades or original color palette McNemar's exact chi-squared test was employed to analyze the confidence level results for each survey subgroup (**Table 4**).

Table 4. *Contingency table for confidence levels in a given survey subgroup*

	High/Medium	**Low**
High/Medium	*a*	*b*
Low	*c*	*d*

Our null hypothesis for this test was that the marginal probabilities for the two categories – "high/medium" and "low" – were the same for both the *microshades* and the original color palette. Our alternate hypothesis was that the marginal probabilities for the two categories were not the same and depended on the color palette used in the plot.

Finally, the average percent of "not accessible" plot ratings for both the original plots and microshades plots reviewed by the Deuteranope group were compared to the results of prior CVD accessibility studies.[21,22,23]

Ethical Statement

The survey questions, data collection methods, data management plan, and recruitment methods were all reviewed and approved by the OHSU Institutional Review Board (IRB). On February 21, 2023, the IRB granted final approval under Exempt Category #2 for our project entitled "Microshades Accessibility Project" (STUDY00025203). The principal investigator for this study is Dr. Lisa Karstens.

All participants were informed of the purpose of our study before completing the survey. Participants were provided with a confidentiality statement and asked to confirm their consent at the beginning of the survey. Survey results were collected anonymously and securely stored in REDCap, an OHSU approved secure web application for creating

and managing online surveys and databases in compliance with HIPAA and IRB requirements.[30] None of the personal identifiers specified by HIPAA's Protected Health Information (PHI) were recorded in connection with survey responses.[42] In order to be eligible to participate in the study, all subjects were required to meet the following criteria: 18+ years old, English as first language, and has experience in either biology, microbiome science, or reading taxa bar plots. Please see the Appendix for the full confidentiality statement provided to all participants.

QIIME2 Plug-In

QIIME2 is a popular, widely used data analysis tool in microbiome research with comprehensive documentation and tutorials as well as an extensive, involved community of users. As of 2022, the QIIME YouTube channel has over 2,500 subscribers.[15] Of the top analysis tools, QIIME2 is designed to be user-friendly and accessible to novice programmers, making it widely adopted throughout the microbiome research community. Importantly for our purposes, QIIME2 allows outside developers to build and publish plug-in packages, making it possible for us to directly integrate the *microshades* CVD color palette.[15]

To avoid creating multiple versions of the *microshades* package in different programming languages, we wrote a Python package that runs functions from the *microshades* R package in a background instance of R using commands from the rpy2 Python package.[44] The function in our QIIME2 plug-in is defined as a "visualizer" as the output is the resulting microshades plot as a QIIME2 visualization.[15] The Python scripts have been submitted to the *q2-microshades* repository in the KarstensLab GitHub.[15] All wrapped functions have been tested and verified with default input values to ensure functionality. Time permitting, we plan to request admin approval from QIIME2 for the new plug-in and publish the package to the QIIME2 Library as an official plug-in.[15]

RESULTS

Participant Demographics

Our initial Pilot group required more time to complete the survey than expected. Studies show that surveys longer than 7-8 minutes tend to see a 20% reduction in response rate.[45] However, in order to reach our target estimated statistical power[41], we required at least 6 taxa bar plots in the evaluation section of our survey. After reviewing the Pilot results, we decided to remove 2 of the original 10 taxa bar plots from the survey, reducing the Evaluation Section from 20 questions to 16 questions and the completion time to an estimated 15 minutes. Although our survey required more than the recommended completion time, we were able to exceed our minimum threshold of completed surveys.

Table 5. *Microshades Accessibility Survey completion rates*

Survey Section	Complete	Incomplete
Consent Information Sheet	100%	0%
Demographic Section	96.8%	3.2%
Evaluation Section – Control	83.3%	16.7%
Evaluation Section – Deuteranope	76.5%	23.5%
Evaluation Section – Protanope	75.9%	24.1%
Evaluation Section – Tritanope	69.0%	31.0%
Evaluation Section – Monochrome	90.9%	9.1%
Evaluation Section – CVD	83.3%	16.7%
Total Surveys	**56.2%**	**43.8%**

Over 200 participants provided their informed consent for participation in our study. However, only completed surveys were considered for analysis. While some groups exceeded the 20-participant threshold, we standardized group sizes by adopting the count from the smallest survey subgroup (20 individuals). To achieve this standard survey subgroup size, only the initial 20 completed responses in each survey subgroup were included, with subsequent participants' results excluded from the analysis.

Table 6. *Survey participants by survey subgroup*

Survey Group	Female	Male	Total	Percent Male
Control	10	10	20	50%
Deuteranope	13	7	20	35%
Protanope	12	8	20	40%
Tritanope	17	3	20	15%
Monochrome	13	7	20	35%
CVD	0	10	10	100%
Total Participants	**65**	**45**	**110**	**41%**

Our final analysis dataset comprised 100 participants with full color vision and 10 participants with various types of CVD. **Table 6** displays the distribution of participant sex within each group, with the Tritanope group showing a notable skew, having only 15% male participants. The CVD cohort consisted entirely of male participants, aligning with the fact that CVD predominantly affects males.[46]

Among the 10 CVD participants (***Table 7***), 60% reported a diagnosis of Deuteranomaly, a form of anomalous trichromacy impacting the "red" cone. The prevalence of Deuteranomaly among our CVD participants reflects its status as the most frequently diagnosed color deficiency.[46] Additionally, Protanomaly, affecting the "green" cone, was reported by some participants, while Tritanomaly, a rarer form of CVD affecting the "blue" cone, was identified in one participant.[46] A final CVD participant reported "Other" and indicated "Red/Green Colorblindness," a broad term encompassing both Deuteranope and Protanope CVD categories.[46]

Table 7. Participants with color vision deficiency

CVD Type	Sex at Birth	Percent
Deuteranomaly	Male	60%
Protanomaly	Male	20%
Tritanomaly	Male	10%
Other	Male	10%

("Red/Green Colorblindness")

Accessibility Rating Results

Examining the accessibility rating results in **Figure 10**, we observed that, for the majority of survey subgroups, microshades plots were less frequently rated as "not accessible." This reduction indicates an improvement in plot accessibility with the *microshades* CVD color palette.

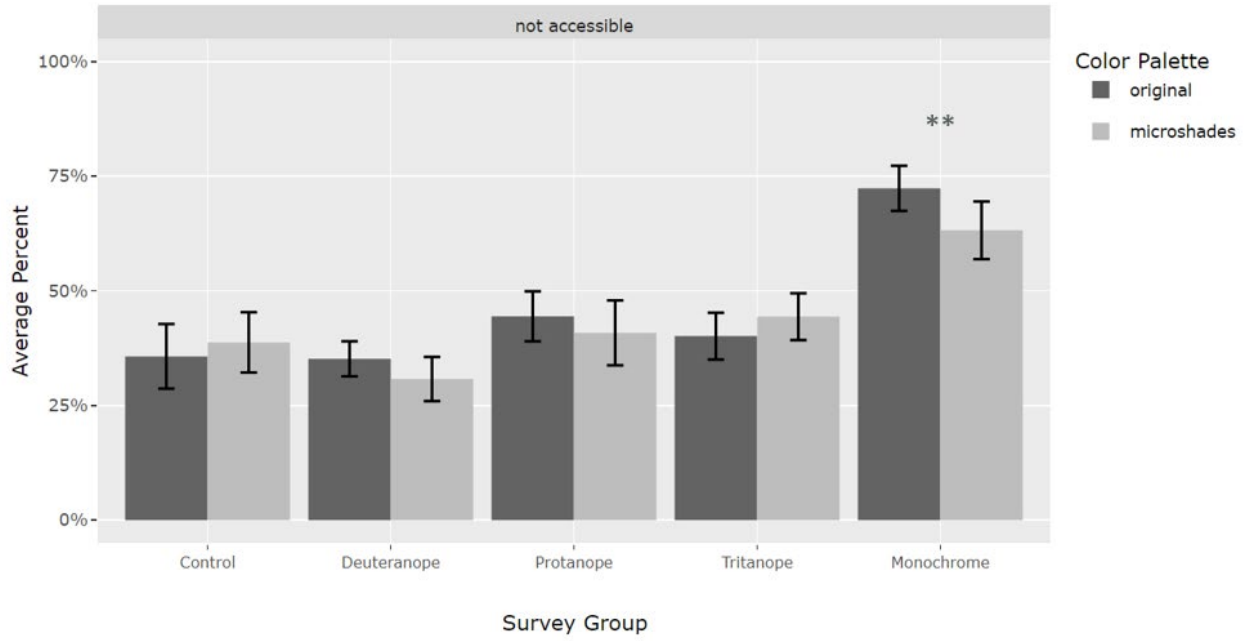

Figure 10. *Percent of plots rated "not accessible" for each participant averaged across survey subgroups of the full color vision cohort*

In **Figure 11**, we see that the accessibility rating results varied between different survey questions. We expected to see fewer participants rating the microshades plot in each question as “not accessible” compared to the original plot. While the results of most questions aligned with our expectations, questions 2, 4, and 7 deviated from our hypothesis. We discuss the unexpected results for these questions in the discussion section below.

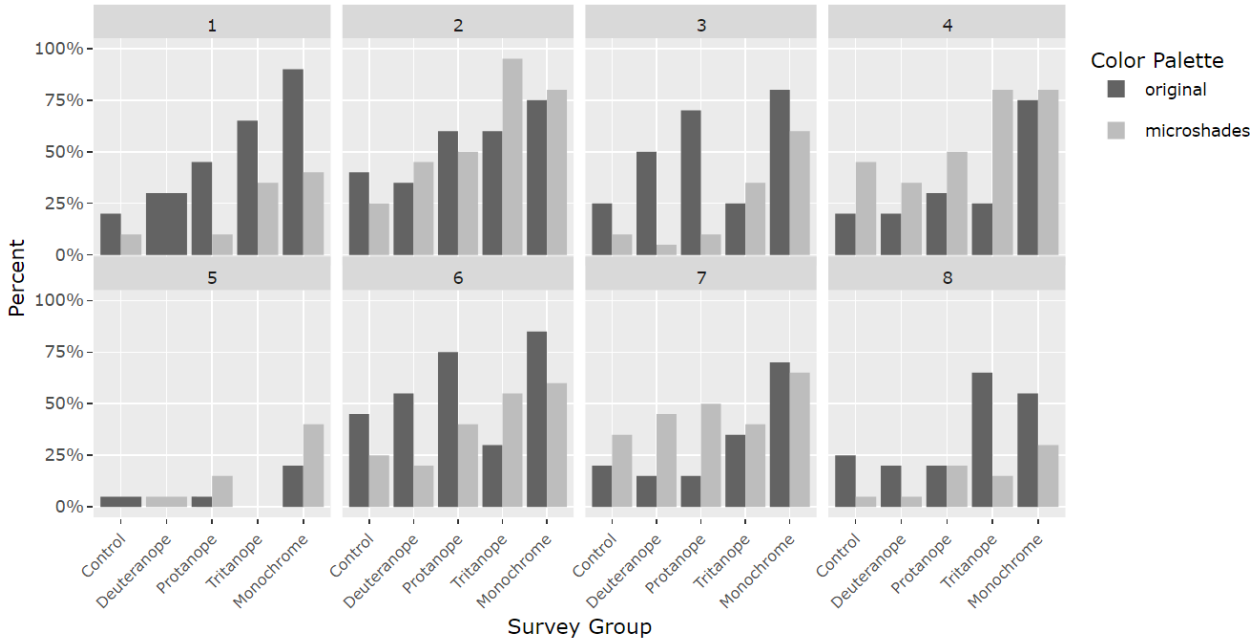

Figure 11. *Percent of full color vision participants rating the plot as “not accessible” for each survey question*

Note: Any “missing” bars in this figure represent a value of 0% (i.e., question 1 – group Deuteranope; question 5 – groups Control, Deuteranope, and Tritanope).

To test our hypothesis, we employed McNemar's exact chi-squared test to analyze the accessibility rating results for each survey subgroup (**Table 8**). By considering binary, paired data, this test compared the number of pairs in agreement between the two categories (accessible/borderline and not accessible) between the original and microshades plots. As shown in both **Figure 10** and **Table 8**, the plots colored with the *microshades* CVD color palette were less frequently rated as "not accessible" when compared to their original counterparts in the following survey subgroups: Deuteranope, Protanope, and Monochrome.

Table 8. *Effects of microshades on plot accessibility*

	Not Accessible				
Survey Group	**Microshades**	**Original**	**Difference**	**Chi Squared**	**p-value**
Control	38.75%	35.71%	3.04%	24	0.20
All Simulated	46.02%	48.61%	-2.59%	120	0.01
Deuteranope	30.77%	35.16%	-4.39%	30	0.08
Protanope	40.83%	44.44%	-3.61%	36	0.06
Tritanope	44.38%	40.13%	4.24%	23	0.23
Monochrome	63.19%	72.37%	-9.17%	31	0.01

Note: Difference = Microshades - Original

The results of our chi-squared test (**Table 8**) found that the *microshades* CVD color palette significantly decreased the number of plots rated as "not accessible" for the “All Simulated” group which combines data from the Deuteranope, Protanope, Tritanope, and Monochrome groups. The Monochrome group, representing the most extreme case of CVD, also showed a significant reduction in the number of plots rated as "not accessible" with the *microshades* CVD color palette. Other survey subgroups – including the Control, Deuteranope, Protanope, and Tritanope groups – did not show a significant difference in accessibility between the microshades and original plots, overall.

Interpretation Accuracy Results

In addition to the accessibility ratings, we also analyzed the participants’ interpretation accuracy. We observed that microshades plots were less frequently interpreted incorrectly across all survey subgroups (**Figure 12**). This improvement in plot readability can be attributed to the *microshades* CVD color palette.

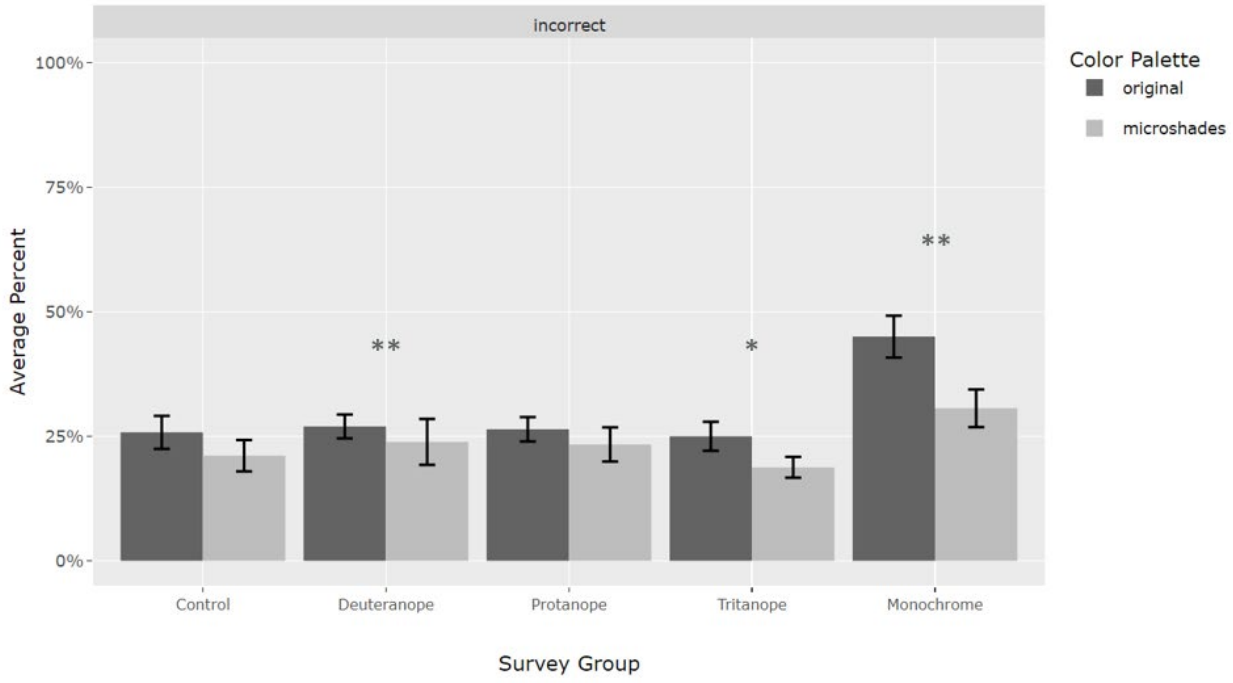

Figure 12. *Percent of plots interpreted incorrectly for each participant averaged across survey subgroups of the full color vision cohort*

As shown in **Figure 13**, the interpretation accuracy varied across survey questions. We expected to see fewer participants interpreting the *microshades* plot incorrectly compared to interpretations of the original plot for each question. While the results of most survey questions aligned with our expectations, questions 4 and 7 deviated from our hypothesis. We discuss the unexpected results for these questions in the Discussion section below.

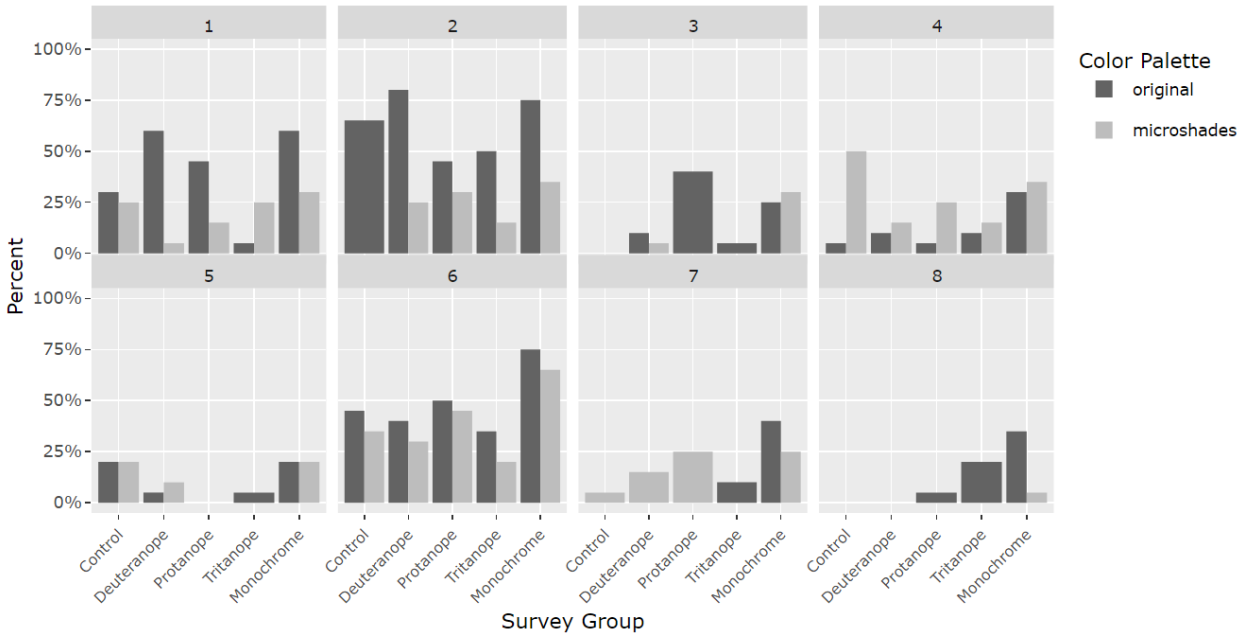

Figure 13. *Percent of participants interpreting the plot incorrectly for each survey question*

Note: Any "missing" bars in this figure represent a value of 0% (i.e., question 2 – group Control; question 3 – groups Control, Protanope, and Tritanope; question 5 – groups Protanope and Tritanope; question 7 – groups Control, Deuteranope, Protanope, and Tritanope; question 8 – groups Control, Deuteranope, Protanope, and Tritanope).

We performed McNemar's exact chi-squared test once again to analyze the interpretation accuracy results for each survey subgroup (**Table 9**). Our analysis found that the *microshades* CVD color palette significantly reduced the number of plots interpreted incorrectly for the agglomerated simulation groups ("All Simulated"). Of the simulation groups, the Deuteranope, Tritanope, and Monochrome groups all showed a significant reduction in the number of plots interpreted incorrectly with the *microshades* CVD color palette as well. The Control and the Protanope groups did not show a significant difference in readability between the microshades and original plots.

Table 9. *Effects of microshades on interpretation accuracy*

	Incorrect				
Survey Group	**Microshades**	**Original**	**Difference**	**Chi Squared**	**p-value**
Control	21.09%	25.78%	-4.69%	19	0.38
All Simulated	25.22%	31.51%	-6.29%	123	<0.001
Deuteranope	23.86%	26.97%	-3.11%	32	0.004
Protanope	23.33%	26.39%	-3.06%	26	0.16
Tritanope	18.75%	25.00%	-6.25%	24	0.04
Monochrome	30.63%	45.00%	-14.37%	41	0.004

Note: Difference = Microshades - Original

Interpretation Confidence Results

Finally, we analyzed the participants' interpretation confidence levels. We observed that *microshades* plots were less frequently interpreted with low confidence levels in the Deuteranope, Protanope, and Monochrome groups (**Figure 14**). However, the average percent of *microshades* plots interpreted with low confidence levels for the Control and Tritanope groups were greater than the average percent for their original counterparts.

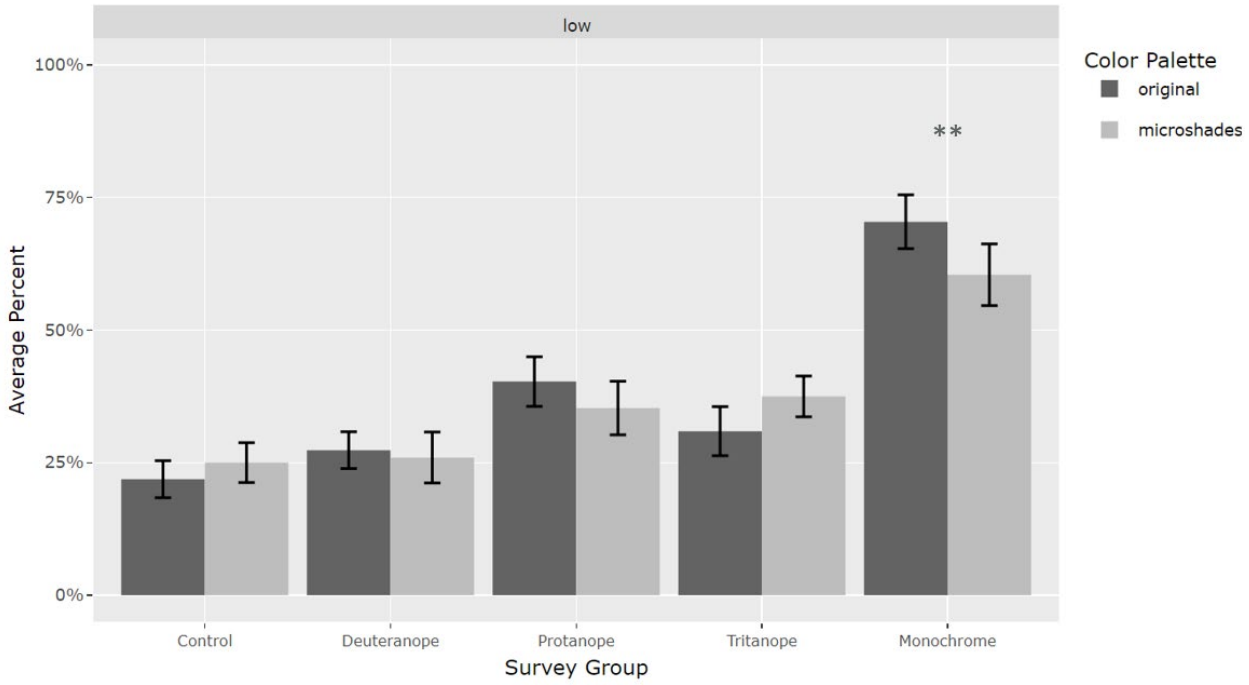

Figure 14. *Percent of plots interpreted with "low" confidence levels (0-3) for each participant averaged across survey subgroups of the full color vision cohort*

As depicted in **Figure 15**, the interpretation confidence results varied between questions. For each question, we expected to see fewer participants interpreting the *microshades* plot with low confidence compared to the original plot. While the results of most survey questions aligned with our expectations, questions 2, 4, and 7 deviated from our hypothesis. We discuss the unexpected results for these questions in the Discussion section below.

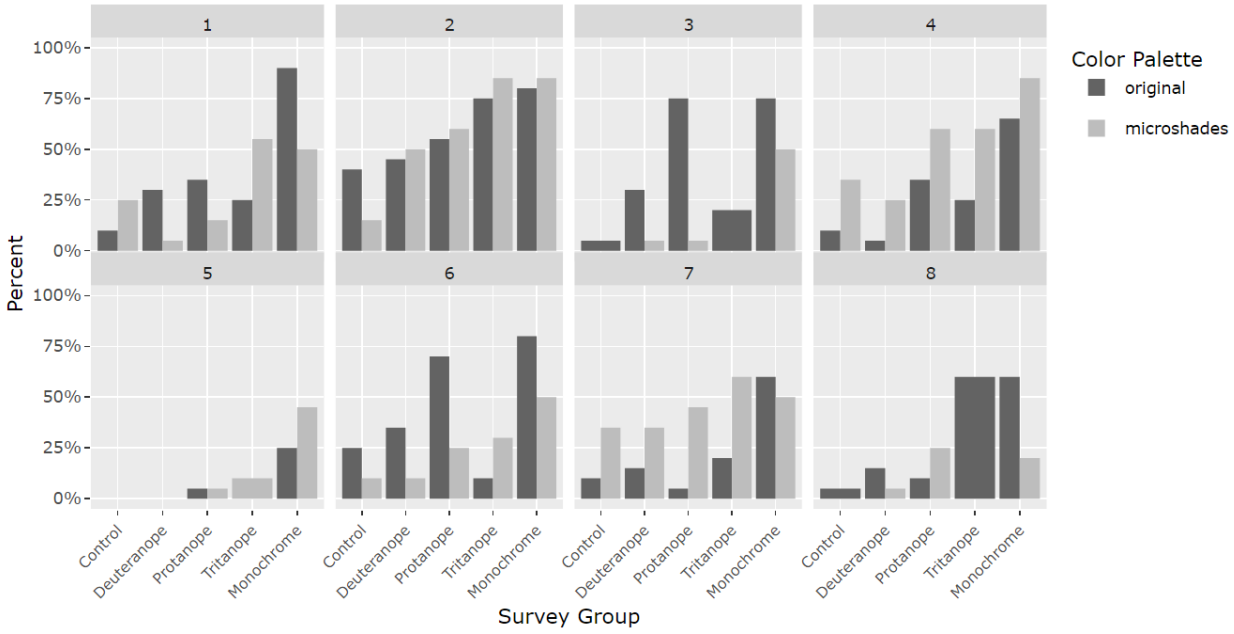

Figure 15. *Percent of participants interpreting the plot with "low" confidence levels for each survey question*

Note: Any "missing" bars in this figure represent a value of 0% (i.e., question 3 – groups Control and Tritanope; question 5 – groups Control, Deuteranope, and Tritanope; question 8 – groups Control and Tritanope).

We performed McNemar's exact chi-squared test once again to analyze the interpretation confidence results for each survey subgroup (**Table 10**). Our analysis found that the *microshades* CVD color palette did not have a significant effect on the number of plots interpreted with low confidence levels for the agglomerated simulation groups ("All Simulated"). Of the simulation groups, only the Monochrome group showed a significant reduction in the number of plots interpreted with low confidence levels in the *microshades* CVD color palette. The Control, Deuteranope, Protanope, and Tritanope groups did not show a significant difference in interpretation confidence between the microshades and original plots.

Table 10. *Effects of microshades on interpretation confidence levels*

	Low Confidence				
Survey Group	**Microshades**	**Original**	**Difference**	**Chi Squared**	**p-value**
Control	25.00%	21.88%	3.12%	15	0.73
All Simulated	40.81%	42.88%	-2.07%	122	0.10
Deuteranope	25.96%	27.34%	-1.38%	26	0.29
Protanope	35.29%	40.28%	-4.98%	37	0.26
Tritanope	37.50%	30.92%	6.58%	23	0.12
Monochrome	60.42%	70.39%	-9.98%	36	0.008

Note: Difference = Microshades - Original

Color Vision Deficient Cohort Results

In addition to the simulated survey results, we also analyzed results from our cohort of CVD participants. Upon initial inspection, the CVD participants' plot accessibility ratings (**Figure 16**) appear to diverge from the results observed in our simulation groups. Surprisingly, we observe an unexpected trend of microshades plots rated "not accessible" more frequently than plots in their original color palettes. This finding warrants further analysis to understand the underlying factors contributing to this discrepancy between the CVD cohort and the simulation groups.

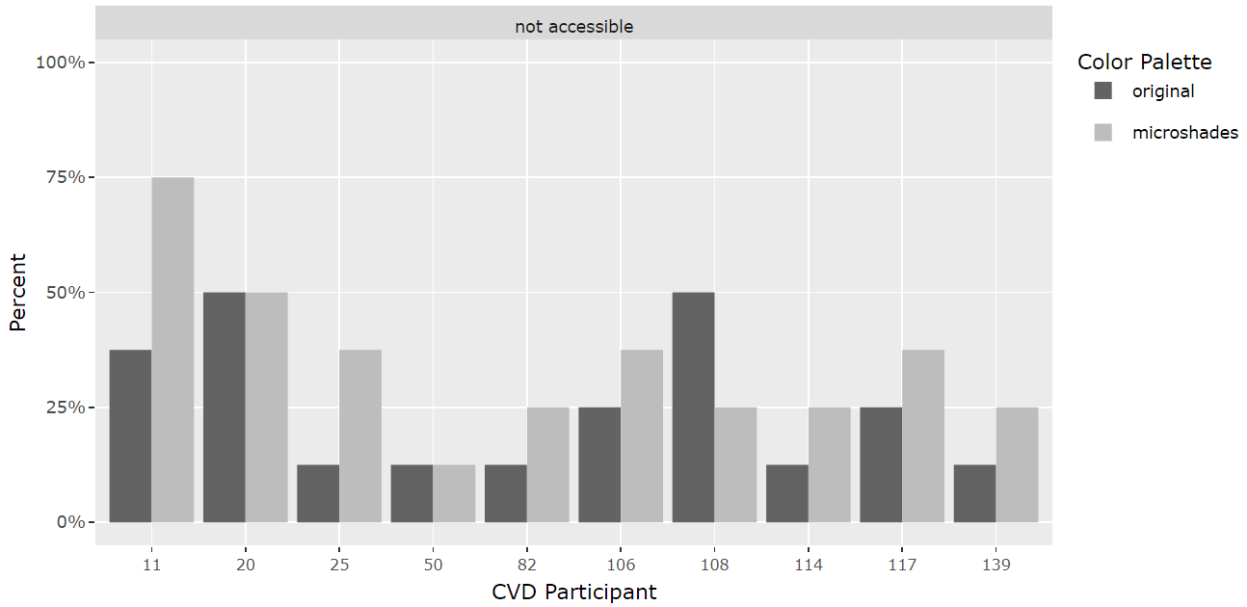

Figure 16. *Percent of plots rated "not accessible" by each CVD participant*

To further analyze the results, we conducted an analysis at a question-specific level. **Figure 17** illustrates the difference in the percent of "not accessible" plot ratings between plots with the *microshades* color palette and the original color palette for both the CVD cohort (in light grey) and the agglomerated simulation groups (in dark grey). As in previous findings, a negative percent difference indicates a reduction in the number of participants rating the plot as "not accessible," indicating improvement in plot accessibility with the *microshades* CVD color palette. The CVD cohort's results varied between questions, mirroring the trends observed in the simulation groups. Both the CVD participants and participants in the simulation groups reported an improvement in plot accessibility with the microshades palette for questions 1, 3, 6, and 8. However, CVD and simulation participants also reported worsening accessibility with *microshades* for questions 2, 4, 5, and 7. Interestingly, both the CVD and simulation groups showed similar trends in terms of directionality of change for each question; however, they differed in magnitude. This difference in magnitude helps to explain the contradictory trends in overall accessibility ratings between the CVD cohort and simulation groups.

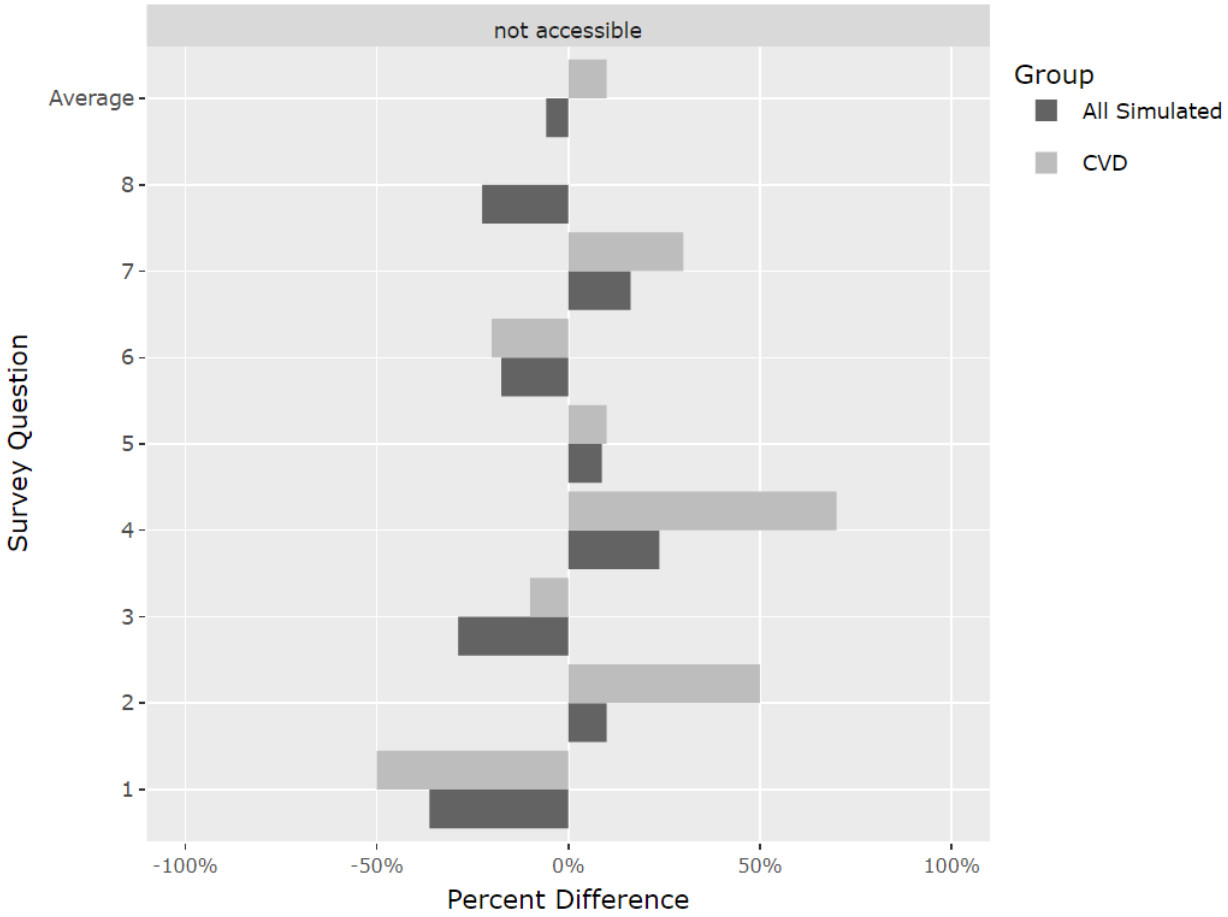

Figure 17. *Comparing effects of microshades on plot accessibility between simulated and CVD groups*

Note: Any "missing" bars in this figure represent a value of 0% (i.e., question 8); a negative percent difference would be interpreted as an improvement in plot accessibility with the microshades color palette.

The CVD participants' plot interpretation accuracy, depicted in **Figure 18** below, more closely aligned with results from our simulation groups. However, this pattern does not uniformly persist across all CVD participants. In the Full Color Vision Cohort, each group exhibited a reduction in the percentage of incorrectly interpreted plots with the *microshades* CVD color palette. These results reveal that a substantial number of CVD participants demonstrated a comparable response to the *microshades* CVD palette's impact on plot readability. However, a portion of CVD participants experienced reduced plot readability when interpreting plots with the *microshades* palette.

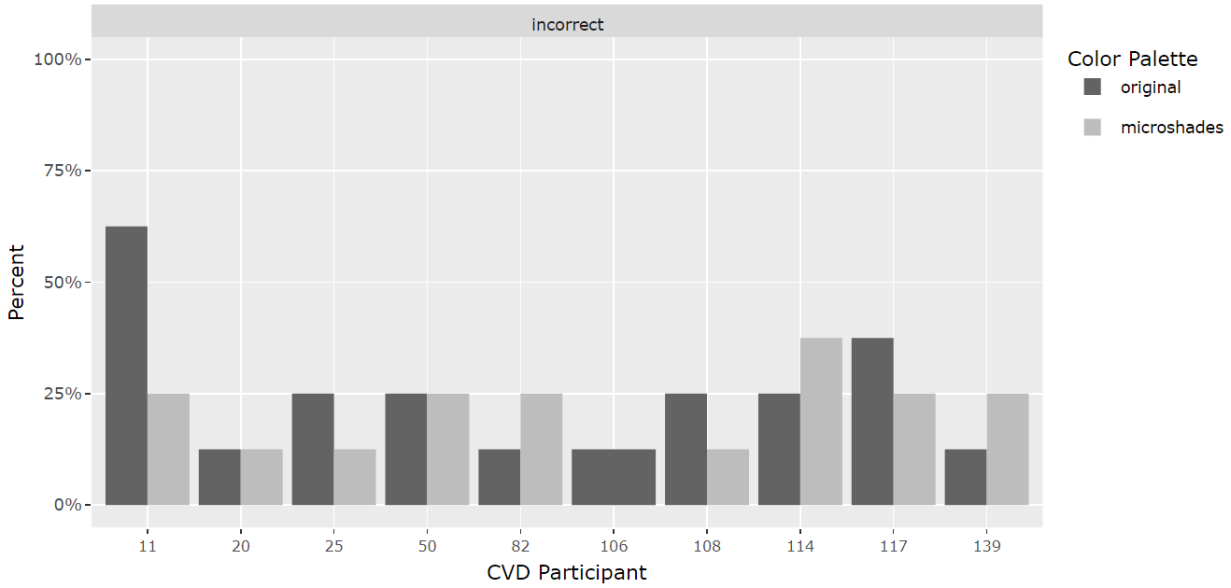

Figure 18. *Percent of plots interpreted incorrectly by each CVD participant*

Note: Any "missing" bars in this figure represent a value of 0% (i.e., participant 106).

Again, we analyzed the CVD cohort results at a question-specific level. As observed with the accessibility results, the CVD cohort's interpretation accuracy results for each question mirrors the patterns seen in the simulation groups. In **Figure 19**, we observe that both the CVD participants and participants in the simulation groups reported an improvement in plot readability with the *microshades* palette for questions 1, 2, 6, and 8. However, CVD and simulation participants also reported worsening readability with *microshades* for questions 4 and 7.

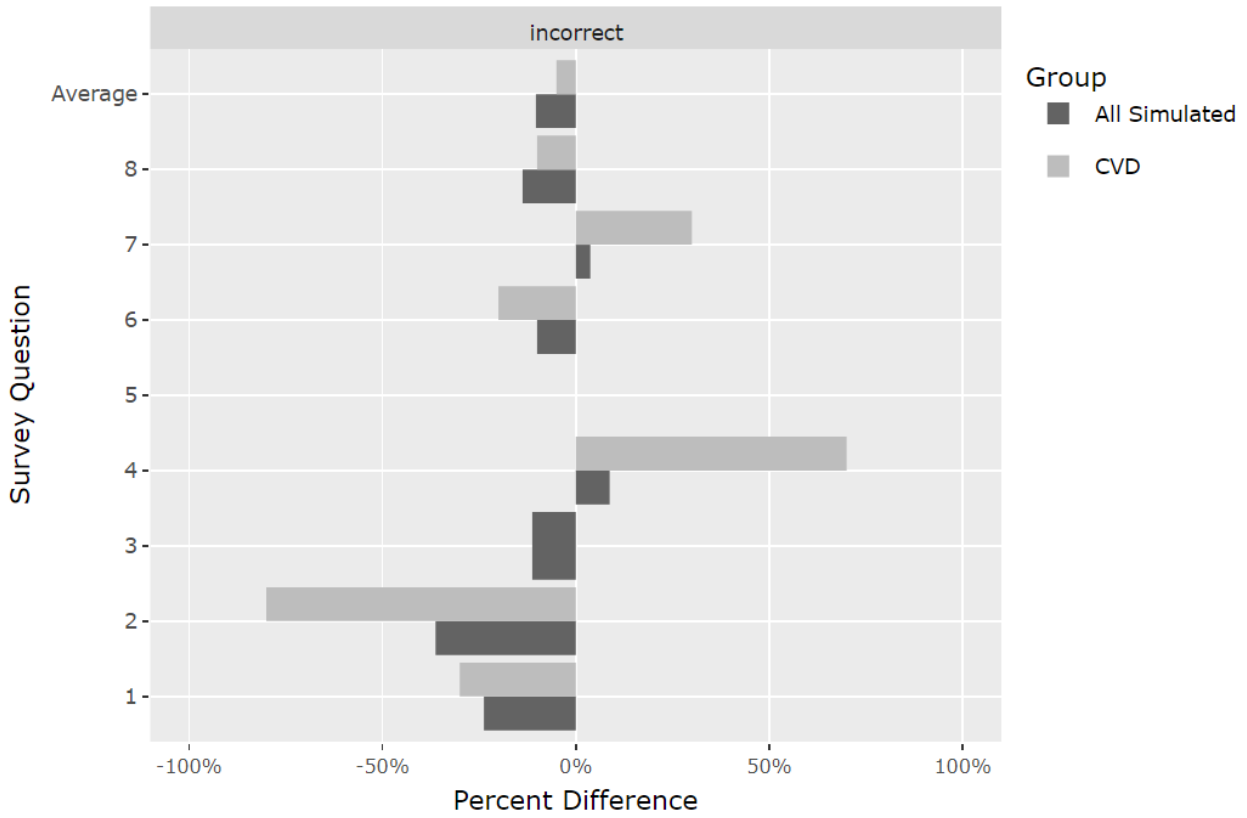

Figure 19. *Comparing effects of microshades on interpretation accuracy between simulated and CVD groups*

Note: Any "missing" bars in this figure represent a value of 0% (i.e., questions 3 and 5); a negative percent difference would be interpreted as an improvement in plot readability with the microshades color palette.

Comparing the effects of the *microshades* CVD color palette on both accessibility and readability between the CVD and simulation participants (**Figure 17** and **Figure 19**), we observed a consistent trend in terms of the directionality of effect. In other words, questions in which the *microshades* color palette improved accessibility or readability for simulation participants also improved that same metric for CVD participants. At the level of individual survey questions, these trends indicate that the CVD simulations are a useful representation of the CVD experience when evaluating plot accessibility and interpretability. However, it must be noted that the accessibility results at the group level did not agree, reinforcing the importance of including CVD participants in any CVD accessibility study. Given the small number of participants in the CVD cohort, we refrained from performing statistical tests.

Table 11. *CVD Cohort accessibility, interpretation accuracy, and confidence results*

Result Metric	Microshades	Original	Difference
Not Accessible	35.00%	25.00%	10.00%
Incorrect	22.22%	25.00%	-2.78%
Low Confidence	23.75%	25.00%	-1.25%

Note: Difference = Microshades – Original; a negative percent difference would be interpreted as an improvement with the microshades color palette.

Survey Questions with Unexpected Results

As mentioned previously, a few questions consistently showed results contrary to the overarching trends and our hypothesized outcomes across both accessibility and readability metrics as well as simulation and CVD participants. In particular, we observed unexpected results with survey questions 2, 4, and 7.

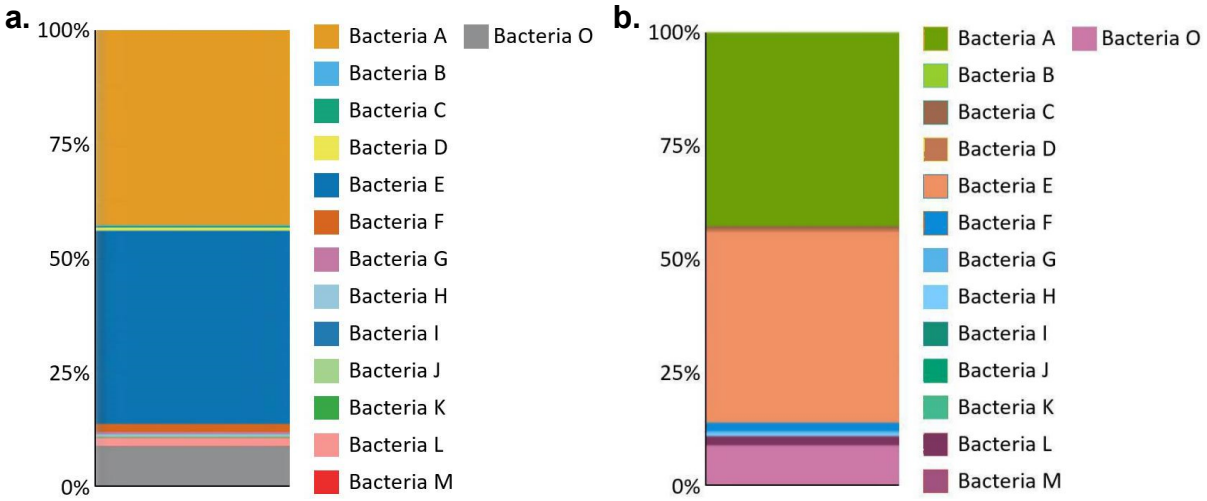

Figure 20. *Survey question #2 taxa bar plot in (a) the original published color palette and (b) the microshades color palette*

Question 2 in our survey asked participants to determine if Bacteria I is present in the sample (**Figure 20**). The correct answer to this question is "No, Bacteria I is not present." With both the simulation and CVD participants, we observed that the *microshades* color palette worsened the plot's accessibility but improved its interpretation accuracy. These

conflicting results can be explained by reviewing the plot's original color palette and the interpretation question posed. For the taxa bar plot in the original published colors, this question is particularly difficult because the authors used two almost identical blue colors to represent two different taxa (Bacteria E and Bacteria I). However, the answer is clear in the *microshades* version of this plot as none of the colors in the palette are repeated. This result highlights the importance of avoiding repeated colors in taxa bar plot palettes.

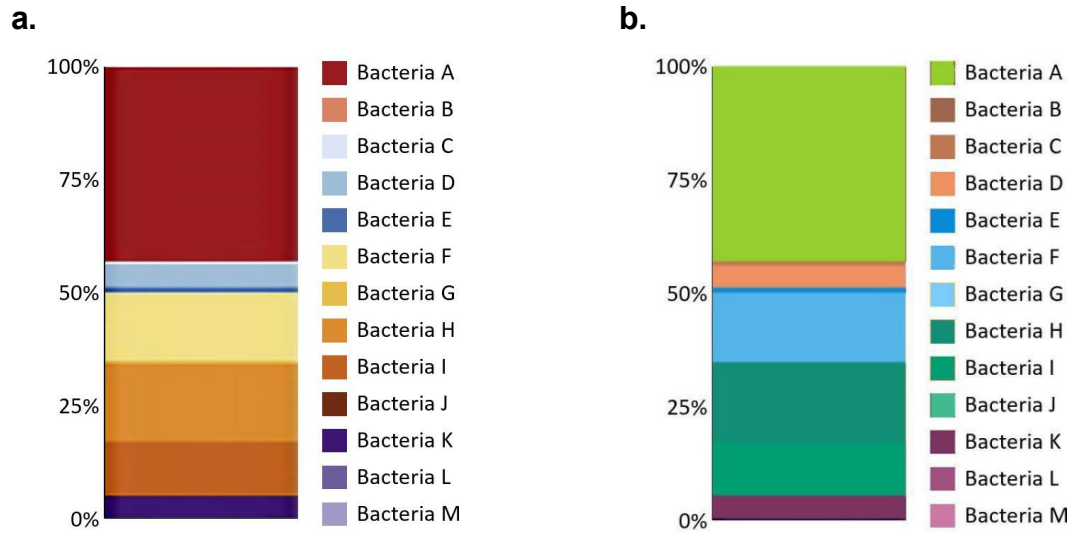

Figure 21. *Survey question #4 taxa bar plot in (a) the original published color palette and (b) the microshades color palette*

Question 4 in our survey asked participants to determine if Bacteria H is present in the sample (**Figure 21**). The correct answer to this question is "Yes, Bacteria H is present." With both the simulation and CVD participants, we observed that the *microshades* color palette worsened the plot's accessibility and interpretation accuracy. These results may also be explained by reviewing the plot's original color palette and the interpretation question posed. In the microshades version of the plot, Bacteria H is represented by a dark teal color, which does not have as much contrast with the adjacent lighter teal color when compared to the colors in the original palette. Unfortunately, the lack of contrast between the adjacent teal colors in the *microshades* version of the plot made it difficult for CVD and simulation participants to interpret, also resulting in lower accessibility ratings. This result highlights the need for a minimum level of contrast between adjacent colors for differentiation.

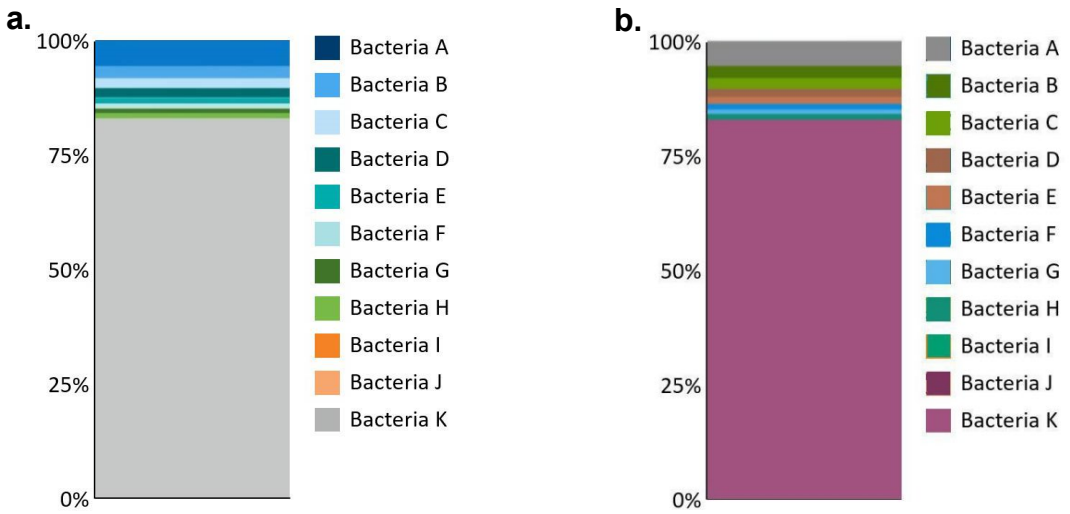

Figure 22. *Survey question #7 taxa bar plot in (a) the original published color palette and (b) the microshades color palette*

Question 7 in our survey asked participants to determine if Bacteria D or Bacteria G is present in greater abundance in the sample (**Figure 22**). The correct answer to this question is "Bacteria D is present in greater abundance." As with survey question 4, we observed that the *microshades* color palette worsened the plot's accessibility and interpretation accuracy for both the simulation and CVD participants. Reviewing the plot's original color palette shed some light on the accessibility results; however, reviewing the interpretation question posed does not necessarily provide a clear explanation for the interpretation results. The original plot's color palette does have greater contrast between colors when compared to those selected for the microshades version of the plot, explaining the worsened accessibility ratings. Regrettably, we do not have a clear explanation for the difference in interpretation accuracy between the color palettes.

DISCUSSION

Microshades Accessibility Survey

Our analysis found that the *microshades* CVD color palette significantly improved both plot accessibility and readability for the agglomerated simulation groups, "All Simulated" (**Table 8** and **Table 9**). This group also demonstrated some improvement in interpretation confidence with the microshades palette; however, the effect size was not substantial enough for significance (**Table 10**). As the "All Simulated" group was a combination of results from the Deuteranope, Protanope, Tritanope, and Monochrome groups, this group had the greatest sample size (n=80). The significance of our findings for both the

plot accessibility and interpretation accuracy with this larger sample size suggests that larger sample sizes in all survey subgroups may have resulted in significant results as well.

In the Deuteranope simulation group, consisting of participants with full color vision who reviewed taxa bar plots with a Deuteranopia CVD simulation, the *microshades* CVD color palette significantly improved plot readability (**Table 9**). However, we found no significant effect on plot accessibility or interpretation confidence with the microshades color palette (**Table 8** and **Table 10**).

Our analysis of the Protanope simulation group, consisting of participants with full color vision who reviewed taxa bar plots with a Protanopia CVD simulation, found that the *microshades* CVD color palette had no significant effect on plot accessibility, readability, or interpretation confidence (**Table 8**, **Table 9**, and **Table 10**).

Similar to the Deuteranope simulation group, the Tritanope simulation group, consisting of participants with full color vision who reviewed taxa bar plots with a Tritanopia CVD simulation, demonstrated significant improvement in plot readability with the *microshades* CVD color palette (**Table 9**). However, we found no significant effect on plot accessibility or interpretation confidence.

Of the four simulation groups, the Monochrome simulation group, consisting of participants with full color vision who reviewed taxa bar plots with a Monochrome/ Achromatopsia CVD simulation, showed the most significant results. Our analysis of this group found that the *microshades* CVD color palette significantly improved results across all metrics, including significant improvements in accessibility, readability, and interpretation confidence, with the microshades palette (**Table 8**, **Table 9**, and **Table 10**). These results indicate that, in the most extreme case of CVD, the microshades palette had the greatest effect and is an improvement over default color palettes currently used in microbiome publications.

As expected, the Control group, consisting of participants with full color vision who reviewed taxa bar plots without CVD simulation, did not show significant improvements in plot accessibility, readability, or interpretation confidence with the *microshades* color palette (**Table 8**, **Table 9**, and **Table 10**). We hypothesized that the effect size for this survey subgroup would not be large enough to significantly detect improvement. Our results support this hypothesis.

The CVD cohort, consisting of participants with various CVD diagnoses who reviewed taxa bar plots without CVD simulation, did not show trends indicating improved plot accessibility with the *microshades* CVD color palette (**Table 11**). However, this cohort did demonstrate small improvements in plot readability and interpretation confidence. Nevertheless, the similarity in the direction of change between the CVD and simulation groups, albeit varying in magnitude, suggests that the *microshades* color palette may still improve these metrics for the larger CVD population as a whole.

Limitations & Future Research

One limitation of this study is the imperfection of CVD simulations.[35] They are a close replication of what someone with CVD would see, but deficiencies in color vision fall on a spectrum and vary from person to person. Our survey-based approach demonstrated that the *microshades* color palette can enhance plot accessibility and interpretation accuracy for individuals with full color vision when reviewing taxa bar plots with CVD simulations. However, the improvement was less pronounced for most CVD participants, possibly due to the limited number of participants in this cohort. Consequently, differences in the magnitude of change between the original color palette and microshades plots contributed to incongruent outcomes overall between the CVD and simulation groups. Replicating the study with CVD participants, not simulations, and focusing on the most common CVD types would strengthen the findings. Increasing the sample size would also enhance the statistical power of the study.

Additionally, limiting the variability between survey questions by generating taxa bar plots from raw data with the same default color palette for original images and the *microshades* package for recolored images would enhance consistency and potentially increase the effect size of improvements with the *microshades* CVD color palette, also increasing the analyses' statistical power and allowing us to detect a significant difference between the color palettes. This study focused solely on the *microshades* CVD color palette and did not explore the entire package itself. Plots were recolored with colors from the microshades palette as images edited using the *imager* package in R, not the *microshades* package. However, if plots were generated from, for example, a phyloseq object, future studies could include scientific figures created with both the *microshades* CVD color palette and the *microshades* color organization formatting.

Our study also could not control for the screen size and settings used when completing the survey. This may have inserted additional variability in the colors and resolution of plots reviewed by subjects.

Finally, our study used JPEG images, which may introduce visual artifacts.[47] Future research should explore using PNG images for more accurate results. Overall, this study's findings underscore the importance of accessible visualizations in scientific research and point to the potential of the *microshades* color palette in improving accessibility of more complex scientific figures.

SUMMARY & CONCLUSIONS

Microshades Accessibility Survey

Based on the results of our Microshades Accessibility Survey and subsequent analysis, we acknowledge the improvement in plot accessibility and interpretation accuracy achieved through the use of the *microshades* CVD color palette for full color vision

participants reviewing CVD simulated taxa bar plots. However, we also recognize that the impact was less pronounced for most CVD participants, possibly due to the lower-than-expected effect size and limited number of participants diminishing the statistical power. Our findings emphasize the importance of accessible visualizations in scientific publications and warrant further investigation into the practical implementation of the *microshades* CVD color palette in accessibility studies.

QIIME2 Plug-In

The functionality of the QIIME2 plug-in developed for this thesis is beyond the original proposal for this package. We aimed to include solely the *microshades* CVD color palette; however, the final package includes additional functionality from the original *microshades* R package. All Python wrapped functions of the *microshades* QIIME2 plug-in have been developed and tested for all default input values in Python. A pull request has been submitted to add our *microshades* QIIME2 plug-in to the *q2-microshades* repository in the KarstensLab GitHub.

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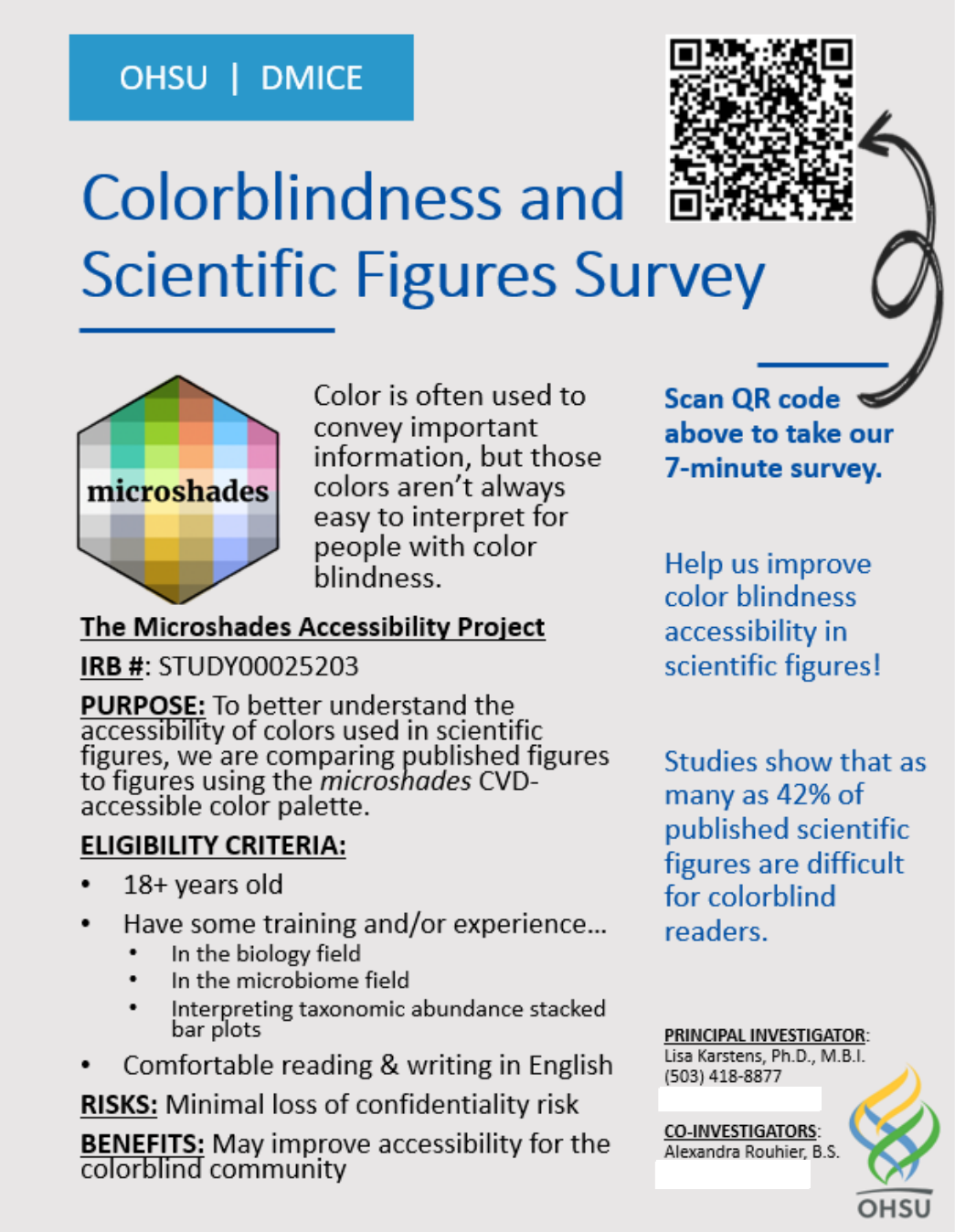

OHSU | DMICE
Colorblindness and Scientific Figures Survey
microshades
Color is often used to convey important information, but those colors aren't always easy to interpret for people with color blindness.
Scan QR code above to take our 7-minute survey.
The Microshades Accessibility Project
IRB #: STUDY00025203
PURPOSE: To better understand the accessibility of colors used in scientific figures, we are comparing published figures to figures using the microshades CVD-accessible color palette.
ELIGIBILITY CRITERIA:
18+ years old
Have some training and/or experience...
In the biology field
In the microbiome field
Interpreting taxonomic abundance stacked bar plots
Comfortable reading & writing in English
RISKS: Minimal loss of confidentiality risk
BENEFITS: May improve accessibility for the colorblind community
Help us improve color blindness accessibility in scientific figures!
Studies show that as many as 42% of published scientific figures are difficult for colorblind readers.
PRINCIPAL INVESTIGATOR:
Lisa Karstens, Ph.D., M.B.I.
(503) 418-8877
CO-INVESTIGATORS:
Alexandra Rouhier, B.S.
OHSU

Subject Line:

OHSU Research - Quick Survey to Study Colorblindness and Scientific Figures

Message:

The Microshades Accessibility Project

IRB #: TBD

PRINCIPAL INVESTIGATOR: Lisa Karstens, Ph.D., M.B.I.

CO-INVESTIGATORS: Alexandra Rouhier, B.S.

PURPOSE:

Color is often used to convey important information, but those colors aren't always easy to interpret for people with color blindness. Studies show that as many as 42% of scientific figures are difficult for colorblind readers to interpret.[1] To better understand the accessibility of colors used in scientific figures, we are comparing published figures to figures using the *microshades* CVD-accessible color palette.

Help us improve color blindness accessibility in scientific figures by completing this 7-minute survey:

https://redcap.link/siospcn5

If you have any questions about our research or the *microshades* package, please feel free to reach out to the study's principal investigator:

Dr. Lisa Karstens
Department of Medical Informatics & Clinical Epidemiology
Oregon Health & Science University

See the attached flier for more details including study Risks and Benefits.

Thanks for your time!

The Karstens Lab

1. Angerbauer K et al. Accessibility for color vision deficiencies: challenges and findings of a large scale study on paper figures. CHI Conference on Human Factors in Computing Systems. 2022 Apr 27.

Do you have 7 minutes? Help us learn about color accessibility in #microbiome science by completing this survey! https://redcap.link/siospcn5 #colorblindness #colordeficient #protanopia #tritanopia #deuteranopia

Survey alert! Help us learn about CVD accessibility of #microbiome data by completing this 7-minute questionnaire: https://redcap.link/siospcn5 #colorblindness #colordeficient #protanopia #tritanopia #deuteranopia

Have #colorblindness? Help us understand accessibility of #microbiome figures by taking this 7-minute survey: https://redcap.link/siospcn5 #colordeficient #protanopia #tritanopia #deuteranopia

OREGON HEALTH & SCIENCE UNIVERSITY

IRB MEMO

Research Integrity Office
3181 SW Sam Jackson Park Road - L106RI
Portland, OR 97239-3098
(503)494-7887 irb@ohsu.edu

APPROVAL OF SUBMISSION

February 21, 2023

Dear Investigator:

On 2/21/2023, the IRB reviewed the following submission:

IRB ID:	STUDY00025203
Type of Review:	Initial Study
Title of Study:	Microshades Accessibility Project
Principal Investigator:	Lisa Karstens
Funding:	None
IND, IDE, or HDE:	None
Documents Reviewed:	• Microshades Accessibility Project - EvaluationSectionCVD_OCTRI13295M.pdf • Microshades Accessibility Project - Waiver or Alteration of HIPAA Authorization.docx • Microshades Accessibility Project - Agreement to Participate.pdf • Microshades Accessibility Project - Consent Information Sheet (1).pdf • Microshades Accessibility Project - DemographicSection_OCTRI13295M • Microshades Accessibility Project - Recruitment Email Script.pdf • Microshades Accessibility Project - Recruitment Flier.pdf • Microshades Accessibility Project - Recruitment Tweets.pdf • Microshades Accessibility Project - Study Protocol.docx • Microshades Accessibility Project - Waiver of Documentation of Consent.pdf

The IRB granted final approval on 2/21/2023. The study requires you to submit a check-in before 2/19/2026.

Version Date: 06/30/2016 Page 1 of 2

Review Category: Exempt Category #2

Copies of all approved documents are available in the study's **Final** Documents (far right column under the documents tab) list in the eIRB. Any additional documents that require an IRB signature (e.g. IIAs and IAAs) will be posted when signed. If this applies to your study, you will receive a notification when these additional signed documents are available.

Ongoing IRB submission requirements:

- Six to ten weeks before the eIRB system expiration date, submit a check-in..
- Any changes to the project must be submitted for IRB approval prior to implementation.
- Reportable New Information must be submitted per OHSU policy.
- Submit a check-in to close the study when your research is completed.

Guidelines for Study Conduct

In conducting this study, you are required to follow the guidelines in the document entitled, "Roles and Responsibilities in the Conduct of Research and Administration of Sponsored Projects," as well as all other applicable OHSU IRB Policies and Procedures.

Requirements under HIPAA

If your study involves the collection, use, or disclosure of Protected Health Information (PHI), you must comply with all applicable requirements under HIPAA. See the HIPAA and Research website and the Information Privacy and Security website for more information.

IRB Compliance

The OHSU IRB (FWA00000161; IRB00000471) complies with 45 CFR Part 46, 21 CFR Parts 50 and 56, and other federal and Oregon laws and regulations, as applicable, as well as ICH-GCP codes 3.1-3.4, which outline Responsibilities, Composition, Functions, and Operations, Procedures, and Records of the IRB.

Sincerely,

The OHSU IRB Office

Version Date: 06/30/2016 Page 2 of 2

Microshades Accessibility Project - Agreement to Participate

Please read the following consent information sheet carefully.

If you have questions, please contact the study team.

Lisa Karstens, Ph.D., M.B.I.

Department of Medical Informatics & Clinical Epidemiology

Oregon Health & Sciences University

IRB Approved: 2/21/2023
Approval Expires: 2/19/2026

OREGON HEALTH & SCIENCE UNIVERSITY

Information Sheet

IRB #: STUDY00025203

TITLE: Microshades Accessibility Project

PRINCIPAL INVESTIGATOR: Lisa Karstens, Ph.D., M.B.I.

CO-INVESTIGATORS: Alexandra Rouhier, B.S.

WHY IS THIS STUDY BEING DONE?

The purpose of this study is to better understand the accessiblity of colors used in scientific figures for those with Color Vision Deficiency (CVD), commonly referred to as color blindness, and how that accessibility could be improved with the microshades CVD-accessible color palette.

Your response to our survey, along with the responses of all other participants, will be securely stored in a data repository. This information will be stored indefinitely and may be used or shared for future research studies.

WHAT EXAMS, TESTS AND PROCEDURES ARE INVOLVED IN THIS STUDY?

This study involves a self-administered, anonymous, one-time questionnaire in English. The questionnaire is made up of two sections: a demographic section and an evaluation section. In the demographic section, we will ask you to provide information about any CVD diagnoses and/or other vision impairments you may have. In the evaluation section, you will be shown scientific figures (taxonomic abundance stacked bar plots) and asked to interpret the figures and rate their accessibility. This survey has been designed to take an average of 7 minutes to complete, but this is not a timed test, so please complete the questions at your own pace.

In the future, your survey response may be given to other researchers for other research studies. The information will be labeled as described in the WHO WILL SEE MY PERSONAL INFORMATION? section.

If you have any questions, concerns, or complaints regarding this study now or in the future, or you think you may have been injured or harmed by the study, please contact Dr. Lisa Karstens at

WHAT RISKS CAN I EXPECT FROM TAKING PART IN THIS STUDY?

Based on the information collected in this study, there is minimal risk to subjects. Although we have made every effort to protect your identity, there is a minimal risk of loss of confidentiality.

WHAT ARE THE BENEFITS OF TAKING PART IN THIS STUDY?

You will not directly benefit from being in this study. However, by serving as a participant, you may help us learn how to benefit those with Color Vision Deficiency, or color blindness, in the future.

WHAT ARE THE ALTERNATIVES TO TAKING PART IN THIS STUDY?

You may choose not to be in this study.

WILL I RECEIVE RESULTS FROM THIS STUDY?

We will not share your survey results with you as we will not be collecting any contact information from you in this survey. However, summary data may be published in a scientific journal at the conclusion of this study.

WHO WILL SEE MY PERSONAL INFORMATION?

In this study we are not receiving any identifiable information about you, so there is little chance of breach of confidentiality. If your information goes outside of OHSU, it might not be protected under federal law from being used or further shared. We would like your permission to keep your survey responses indefinitely. As the survey will be completed anonymously, none of your identifying information will be connected to your survey response, and we will not be able to identify your personal survey response if you ever wish to be removed from the dataset. If you have any questions, you can contact us at:

Lisa Karstens, Ph.D.

Department of Medical Informatics & Clinical Epidemiology

Oregon Health & Sciences University

You do not have to allow the use and disclosure of your health information in the study, but if you do not, you cannot be in the study. If you choose not to participate, or if you decide to stop at any time, that will not affect your ability to receive health care at OHSU or insurance coverage.

WILL ANY OF MY INFORMATION OR SAMPLES FROM THIS STUDY BE USED FOR ANY COMMERCIAL PROFIT?

Survey results obtained from you in this research study may be used for commercial purposes, such as making a discovery that could, in the future, be patented or licensed to a company, which could result in a possible financial benefit to that company, OHSU, and its researchers. There are no plans to pay you if this happens. You will not have any property rights or ownership or financial interest in or arising from products or data that may result from your participation in this study. Further, you will have no responsibility or liability for any use that may be made of your information.

WHERE CAN I GET MORE INFORMATION?

This research is being overseen by an Institutional Review Board ("IRB"). You may talk to the IRB at (503) 494-7887 or irb@ohsu.edu if:

- Your questions, concerns, or complaints are not being answered by the research team.
- You want to talk to someone besides the research team.
- You have questions about your rights as a research subject.
- You want to get more information or provide input about this research.

You may also submit a report to the OHSU Integrity Hotline online at https://secure.ethicspoint.com/domain/media/en/gui/18915/index.html or by calling toll-free (877) 733-8313 (anonymous and available 24 hours a day, 7 days a week).

DO I HAVE TO TAKE PART IN THIS STUDY?

You do not have to join this or any research study. If you do join, and later change your mind, you may quit at any time. If you refuse to join or withdraw early from the study, there will be no penalty or loss of any benefits to which you are otherwise entitled.

The participation of OHSU students or employees in OHSU research is completely voluntary and you are free to choose not to serve as a research subject in this protocol for any reason. If you do elect to participate in this study, you may withdraw from the study at any time without affecting your relationship with OHSU, the investigator, the investigator's department, or your grade in any course. If you would like to report a concern with regard to participation of OHSU students or employees in OHSU research, please call the OHSU Integrity Hotline at 1-877-733-8313 (toll free and anonymous).

HOW DO I TELL YOU IF I WANT TO TAKE PART IN THIS STUDY?

Please indicate whether you provide your consent to participate in this study using the check boxes below:

◯ Yes, I agree to participate in this study.
◯ No, I do not agree to participate in this study.

Demographic Section

Survey 2 of 3

This section will take aproximately 3 minutes to complete.

Please complete the survey below.

Thank you!

Demographic Section

Are you a minor (under 18 years old)?

- ◯ Yes
- ◯ No

What is your sex assigned or assumed at birth:

- ◯ Female
- ◯ Male
- ◯ Intersex
- ◯ Not listed above (please specify below)
- ◯ Prefer not to state

If you selected "Not listed above" in the previous question, please enter the assigned or assumed sex that best describes you below.

_____

Is English your first language?

- ◯ Yes
- ◯ No

Please indicate your highest level of education:

- ◯ No schooling completed
- ◯ Primary school
- ◯ Some high school
- ◯ High school or GED graduate
- ◯ Some college credit
- ◯ Trade/technical/vocational training
- ◯ Associate degree
- ◯ Bachelor's degree
- ◯ Master's degree
- ◯ Professional degree (i.e. MD, DMD, DO)
- ◯ Doctorate degree (i.e. PhD)
- ◯ Other (please specify below)

If you selected "Other" in the previous question, please enter your highest level of education below.

_____

Please select one of the folowing options that best describes you:

- ◯ I currently work or have previously worked in the biology field.
- ◯ I currently work or have previously worked in the microbiome field.
- ◯ I currently study or have previously studied biology.
- ◯ I currently study or have previously studied the microbiome.
- ◯ I have experience interpreting taxonomic abundance stacked bar plots.
- ◯ None of the above.

Have you been diagnosed with any type of Color Vision Deficiency (aka: "color blindness" or CVD)?

- ◯ Yes
- ◯ No

Please indicate which type of Color Vision Deficiency best describes your diagnosis.

- ◯ Protanomaly (green colors look more red)
- ◯ Deuteranomaly (red colors look more green)
- ◯ Tritanomaly (hard to tell the difference between blue & green and yellow & red)
- ◯ Protanopia (unable to tell the difference between red & green at all because green colors are seen as red)
- ◯ Deuteranopia (unable to tell the difference between red & green at all because red colors are seen as green)
- ◯ Tritanopia (unable to tell the difference between blue & green, purple & red, and yellow & pink)
- ◯ Achromatopsia (monochromacy, you can't see colors at all)
- ◯ Other (please specify below)

If you selected "Other" in the previous question, please enter your CVD diagnosis below.

_____

Have you been diagnosed with any other vision impairment(s)? If yes, please select all that apply to you. If no, please select "No".

- ☐ Nearsightedness
- ☐ Farsightedness
- ☐ Astigmatism
- ☐ Dyslexia
- ☐ Cataract
- ☐ Macular Degeneration
- ☐ Glaucoma
- ☐ Low vision
- ☐ Blindness
- ☐ Wears prescription glasses or corrective lenses
- ☐ Other (please specify below)
- ☐ No

If you selected "Other" in the previous question, please enter your vision impairment below.

_____

If you indicated having one or more vision impairments in the previous question, please select one of these three options:

- ◯ I confirm that (1) I am wearing corrective lenses which correct for all of my vision impairments, and (2) I will use these corrective lenses for the duration of this survey.
- ◯ I am completing this survey without any corrective lenses.
- ◯ I am completing this survey with corrective lenses which do NOT correct for all of my vision impairments.

Please select one of the options below at random:

- ◯ Orange
- ◯ Green
- ◯ Blue
- ◯ Yellow
- ◯ Purple

How did you hear about this survey?

- ◯ OHSU meeting or conference
- ◯ Other conference
- ◯ Email
- ◯ Twitter
- ◯ LinkedIn
- ◯ Other

Please feel free to write in any other information you think we should know about you:

Evaluation Section - CVD

Survey 3 of 3

This section will take aproximately 12 minutes to complete.

Please complete the survey below.

Thank you!

PLEASE READ THE FOLLOWING
Accessibility is the practice of making information, activities, and/or environments meaningful and usable for as many people as possible.

In this survey, you will be asked to look at 20 scientific plots showing the taxonomic abundance of one sample, and then asked to interpret the plot and rate its accessibility.

If the plot is clear and easy to read, it would be rated as "accessible". If the plot is confusing or difficult to read, it would be rated as "not accessible". Anything in between would be rated as "borderline".

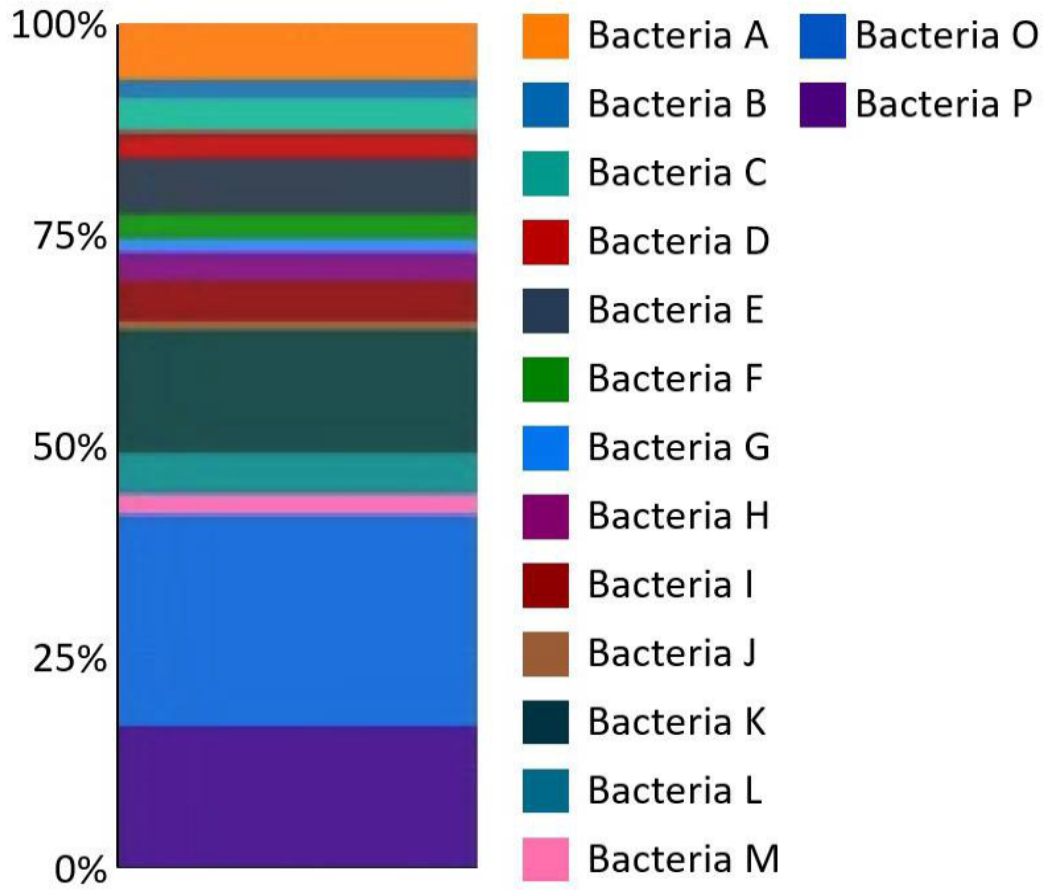
Please indicate that you have read and understood the text above.

Please use the text box below each accessibility question to briefly explain why you chose either "accessible", "borderline", or "not accessible".

◯ Yes, I have read and understood the text above.
◯ No, I have not have read or I do not understand the text above.

Evaluation Question #1

Figure #1



Which taxa is present in greater abundance: Bacteria H or Bacteria I?

◯ Bacteria H
◯ Bacteria I

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

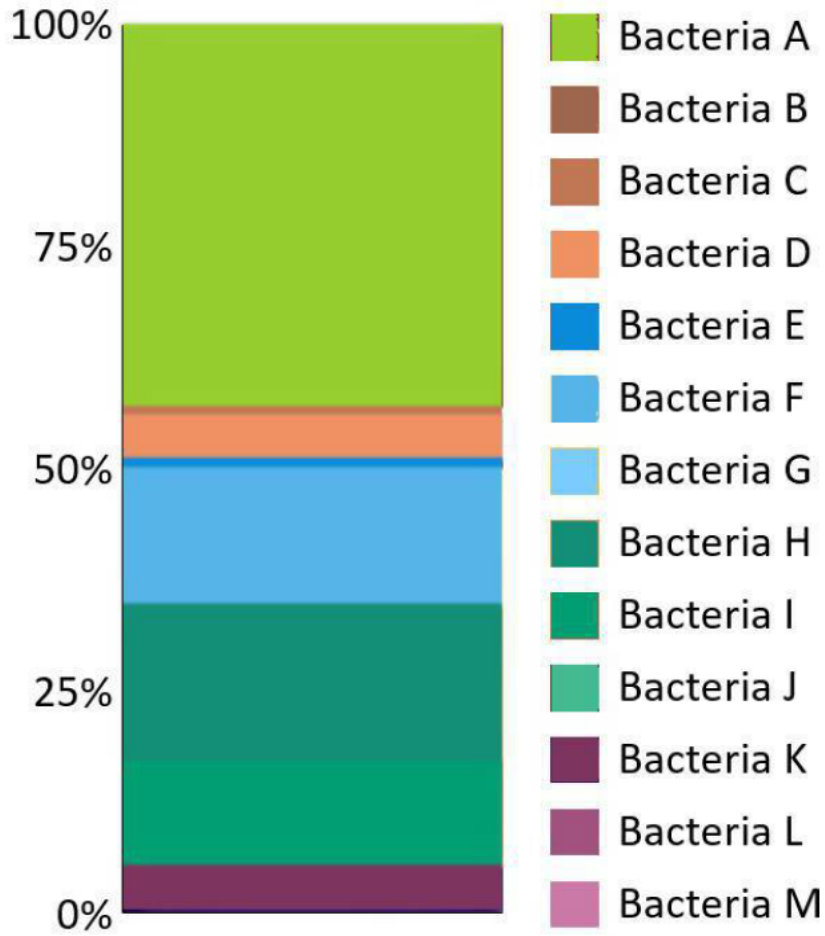
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #2

Figure #2



Is Bacteria H present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

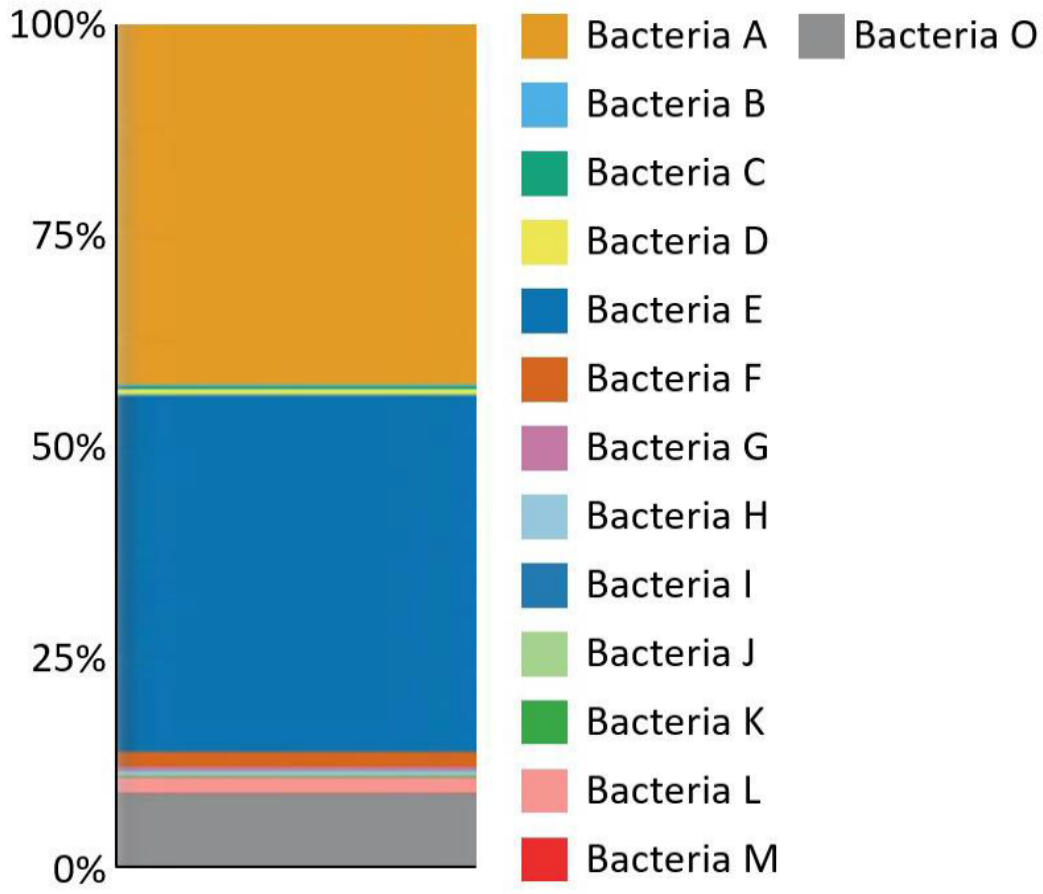
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #3

Figure #3



Is Bacteria I present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

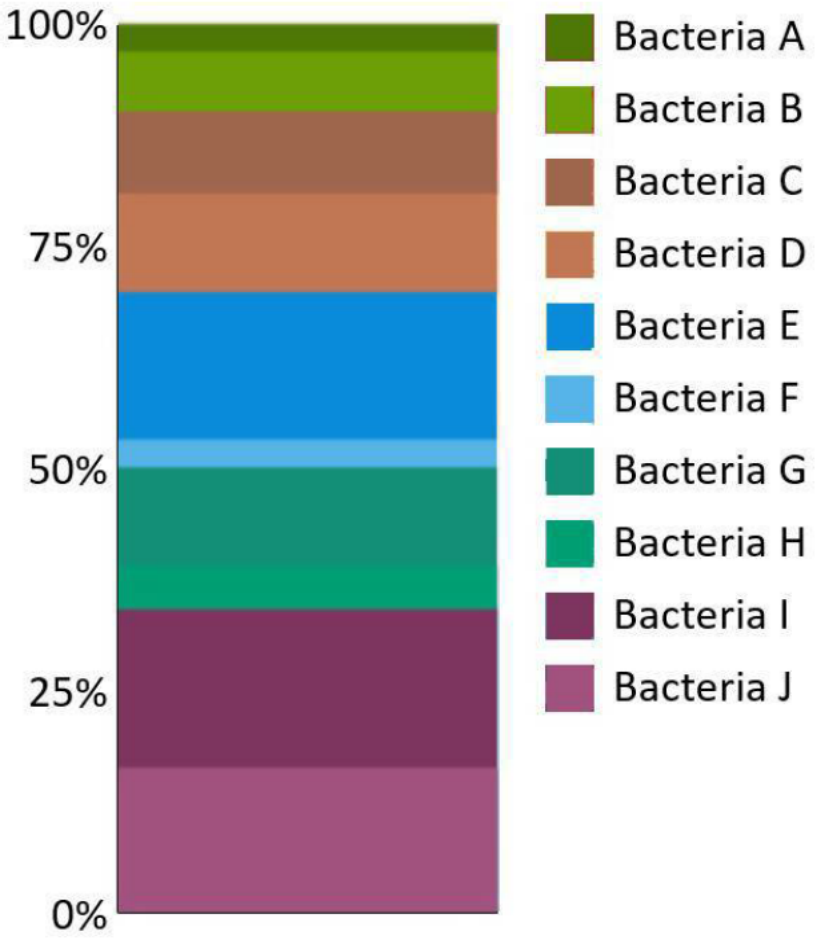
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #4

Figure #4



Which taxa is present in greater abundance: Bacteria D or Bacteria E?

◯ Bacteria D
◯ Bacteria E

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

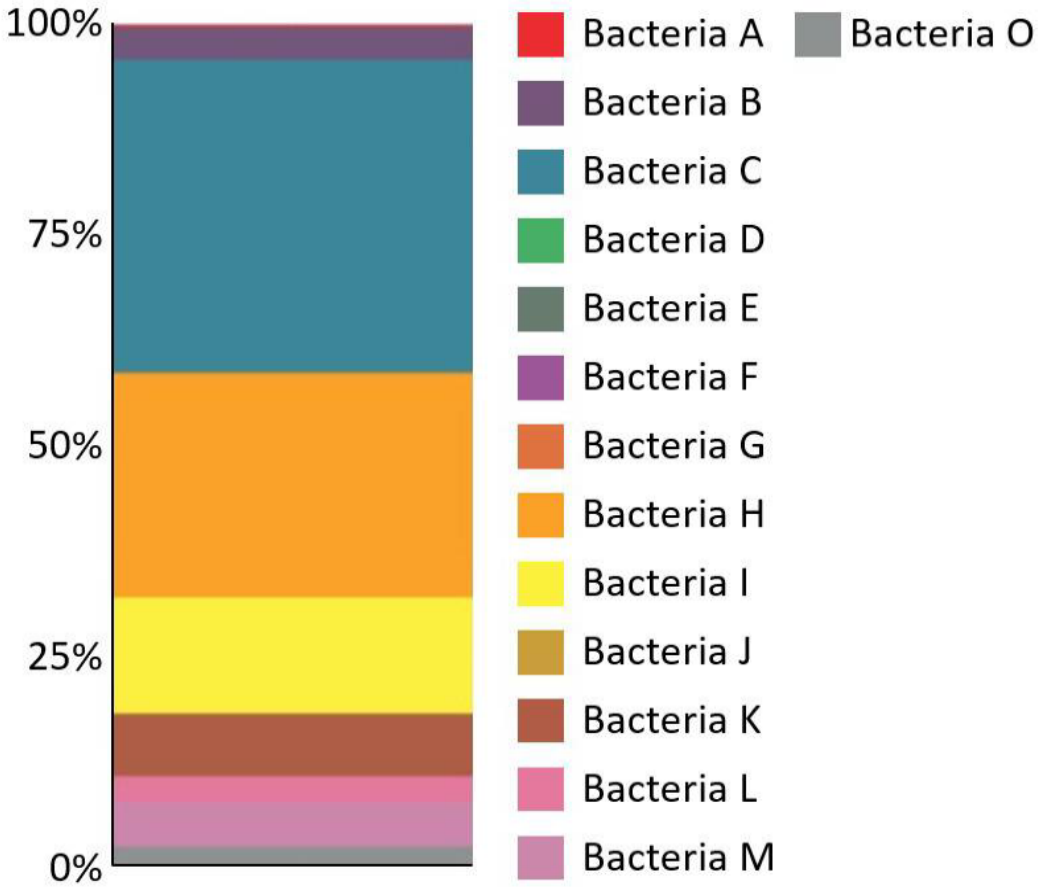
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #5

Figure #5



Is Bacteria L present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

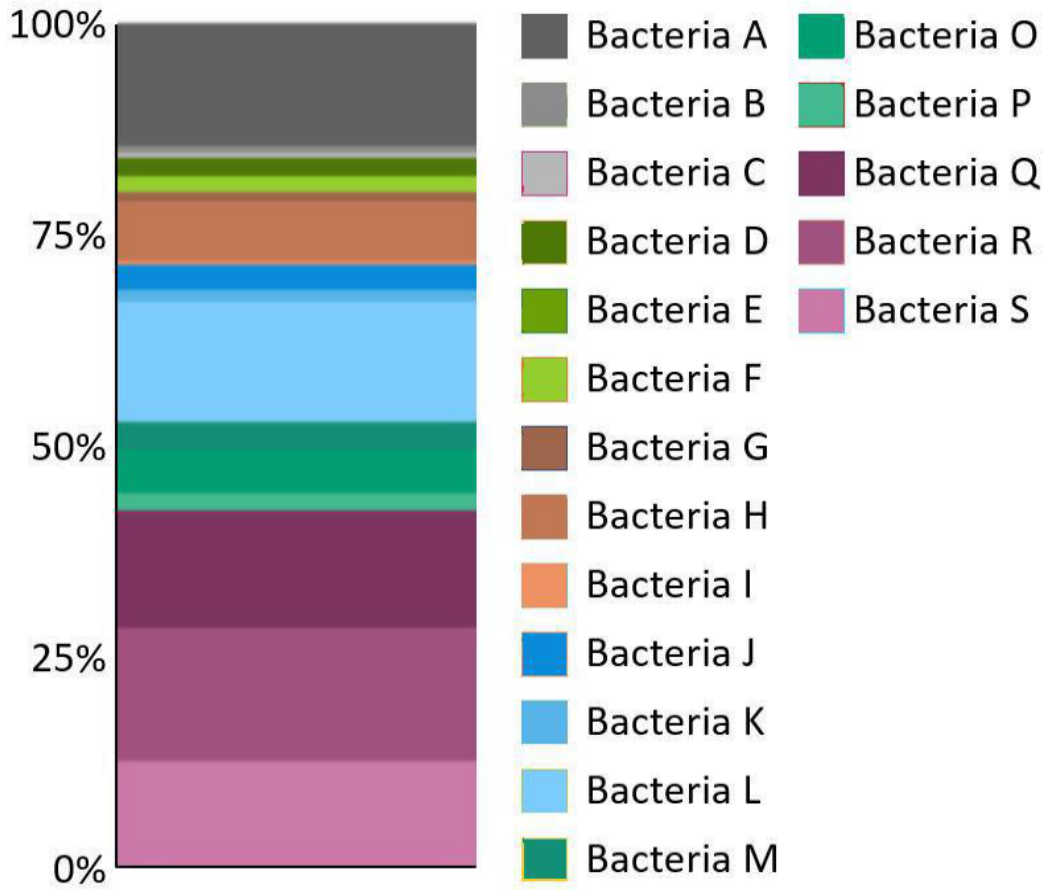
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #6

Figure #6



Which taxa is present in least abundance: Bacteria I or Bacteria J?

◯ Bacteria I
◯ Bacteria J

How confident are you in your previous response?

Not confident	Somewhat confident	Very confident

(Place a mark on the scale above)

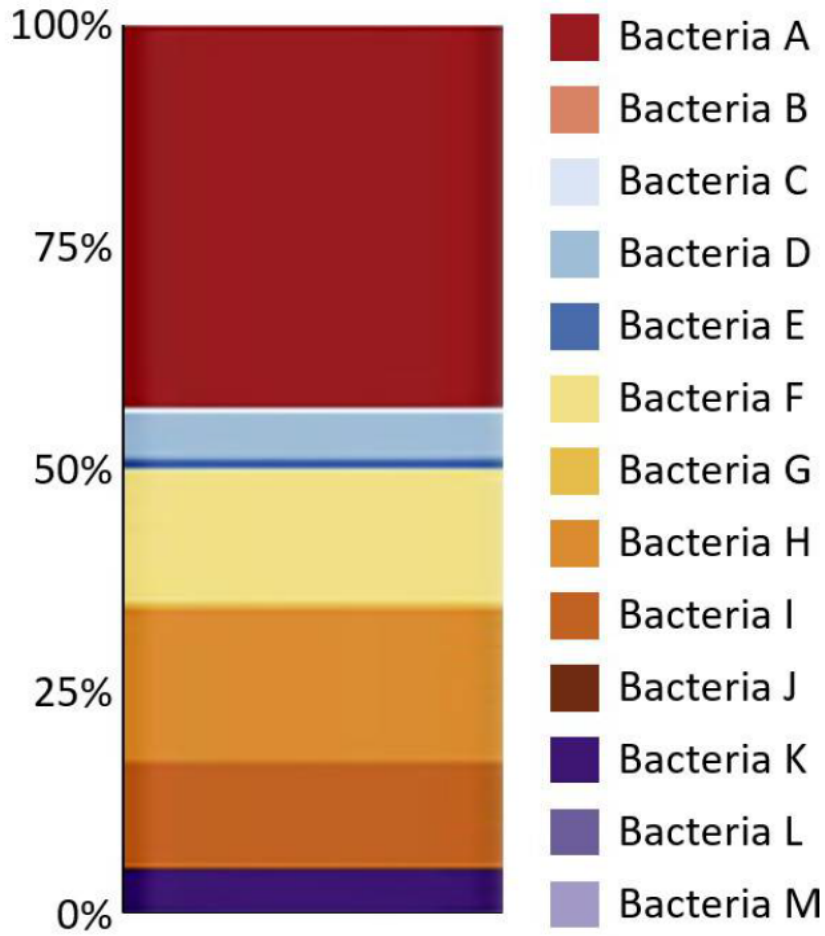
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #7

Figure #7



Is Bacteria H present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

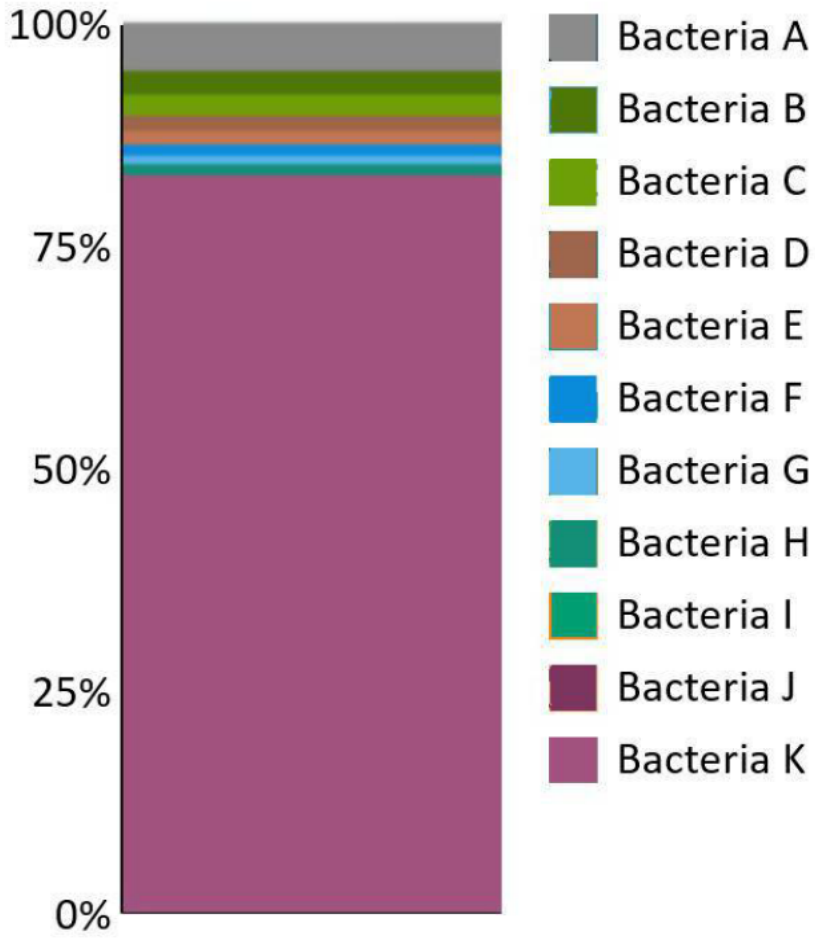
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #8

Figure #8



Which taxa is present in greatest abundance: Bacteria D or Bacteria G?

◯ Bacteria D
◯ Bacteria G

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

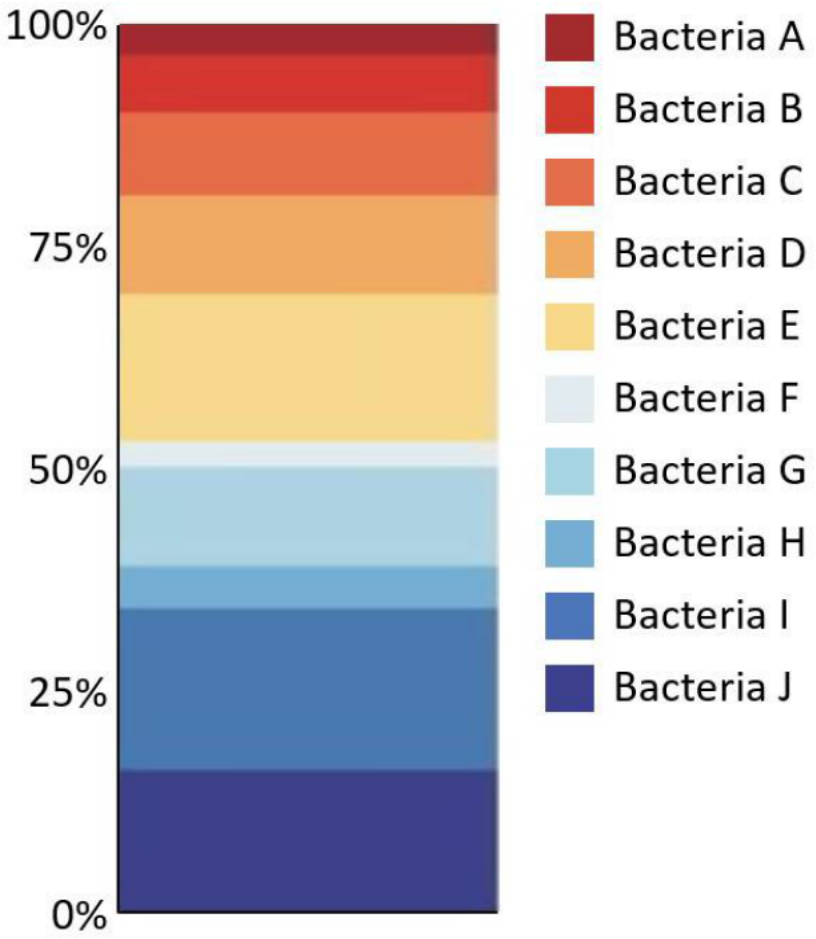
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #9

Figure #9



Which taxa is present in greater abundance: Bacteria D or Bacteria E?

◯ Bacteria D
◯ Bacteria E

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

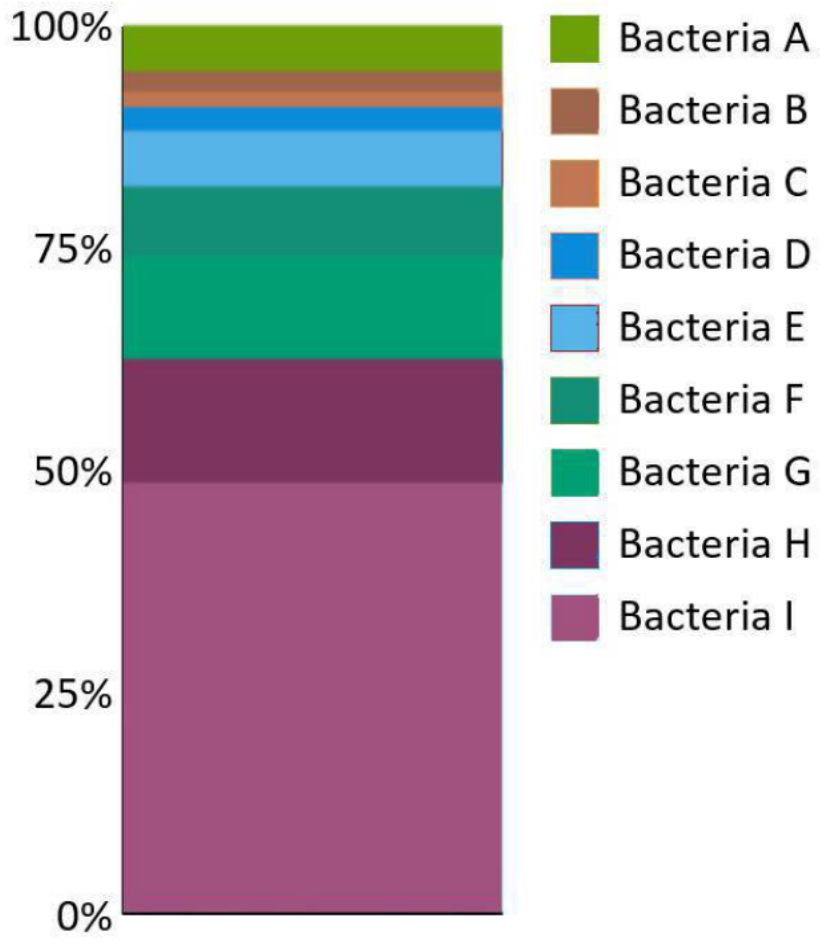
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #10

Figure #10



Is Bacteria C present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

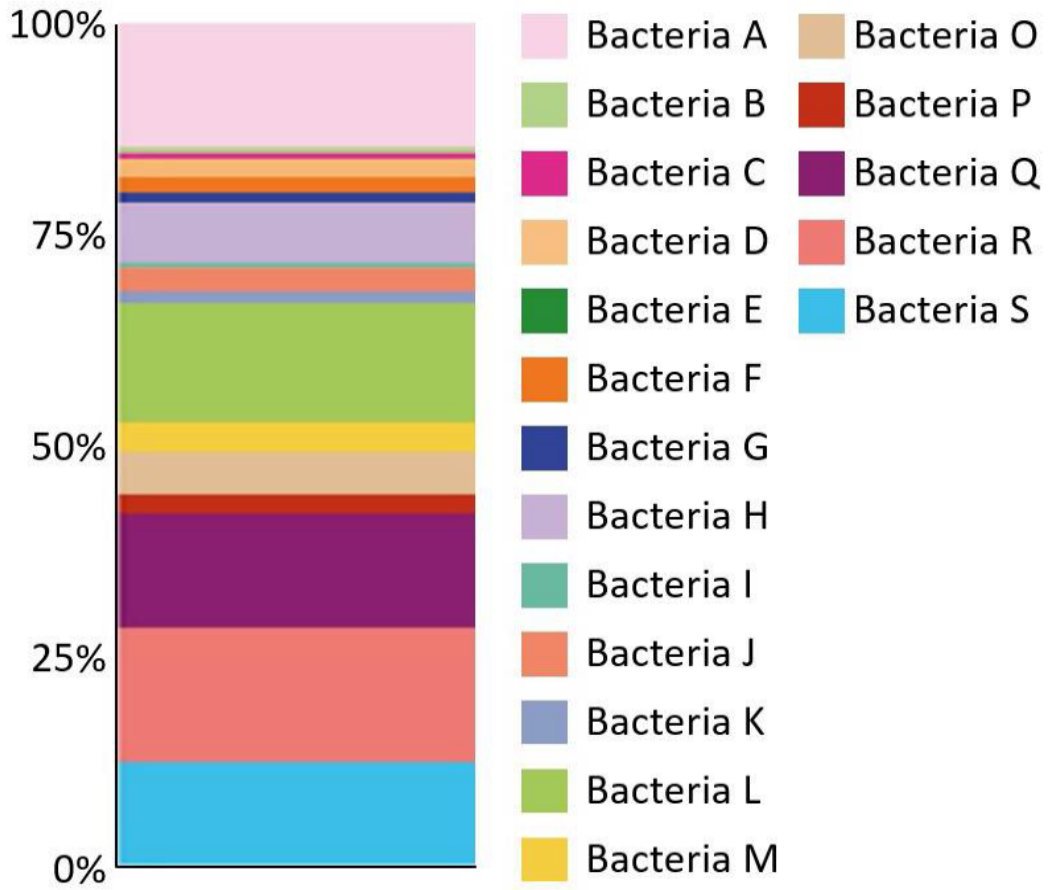
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #11

Figure #11



Which taxa is present in least abundance: Bacteria I or Bacteria J?

◯ Bacteria I
◯ Bacteria J

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

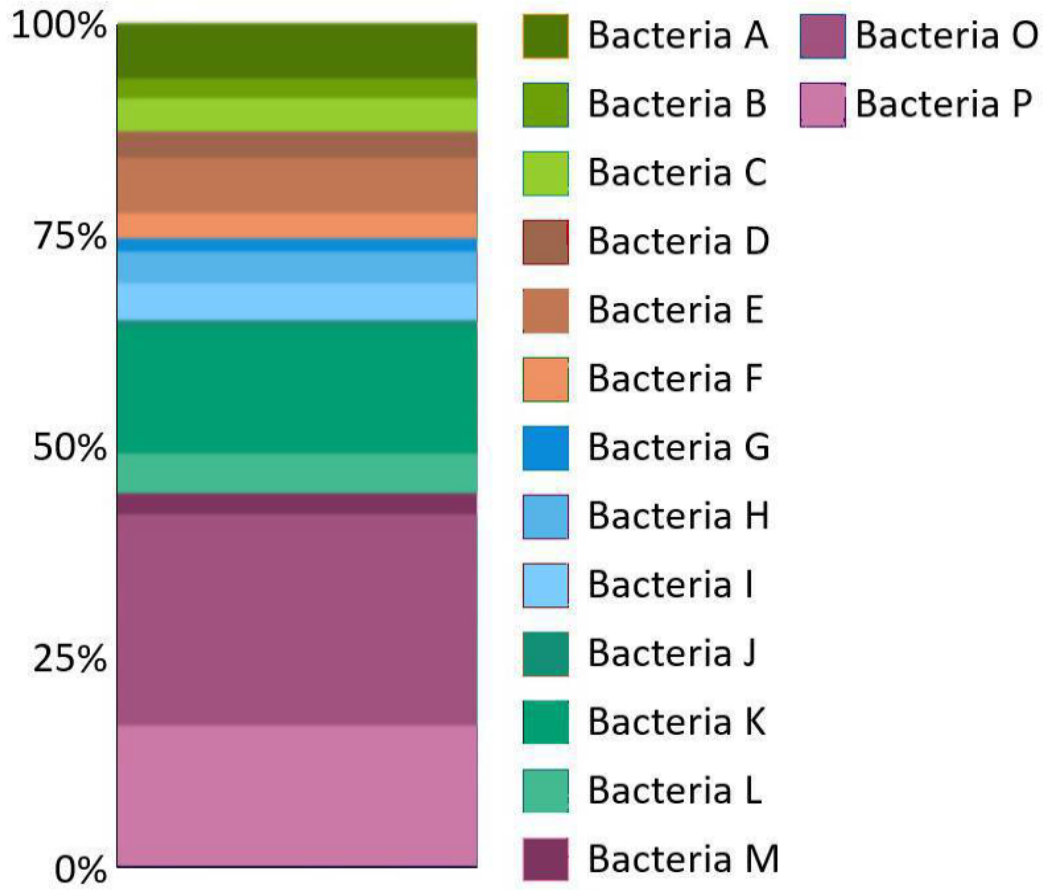
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #12

Figure #12



Which taxa is present in greater abundance: Bacteria H or Bacteria I?

◯ Bacteria H
◯ Bacteria I

How confident are you in your previous response?

Not confident	Somewhat confident	Very confident

(Place a mark on the scale above)

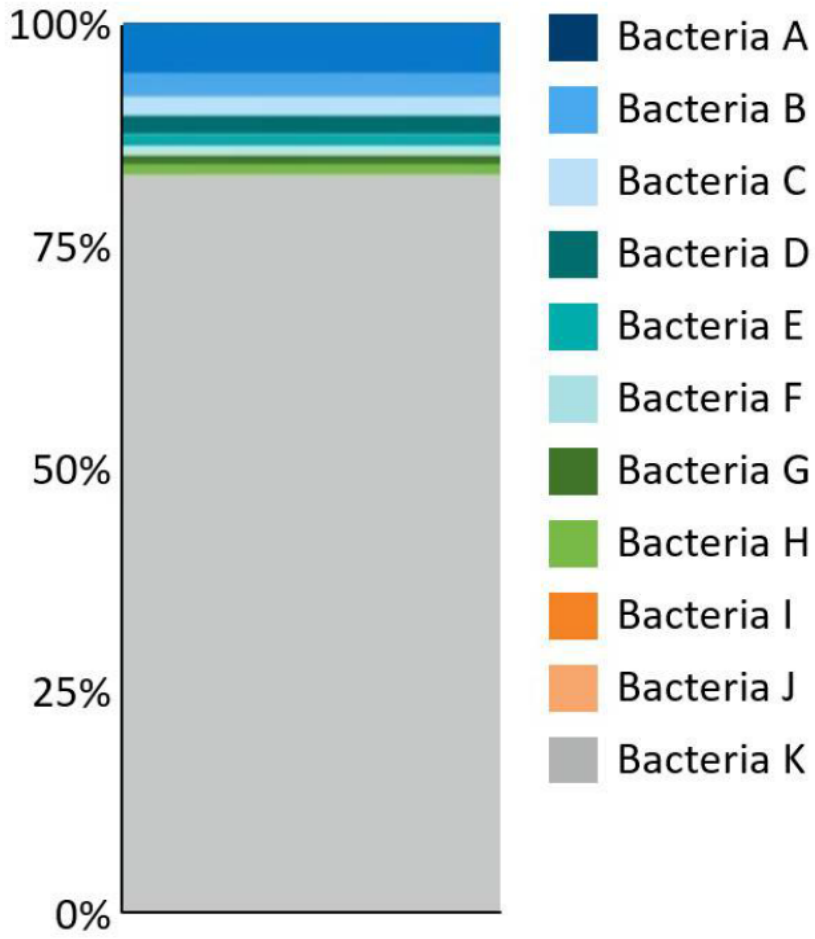
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #13

Figure #13



Which taxa is present in greatest abundance: Bacteria D or Bacteria G?

◯ Bacteria D
◯ Bacteria G

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

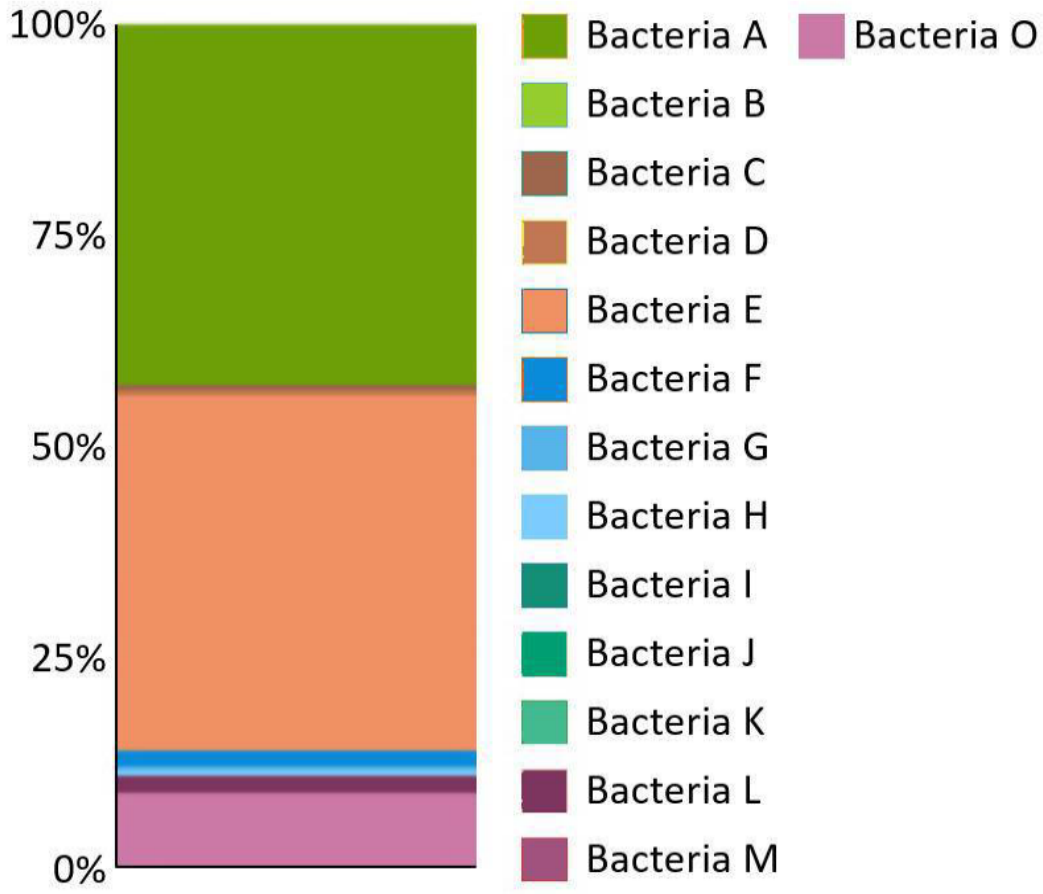
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #14

Figure #14



Is Bacteria I present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

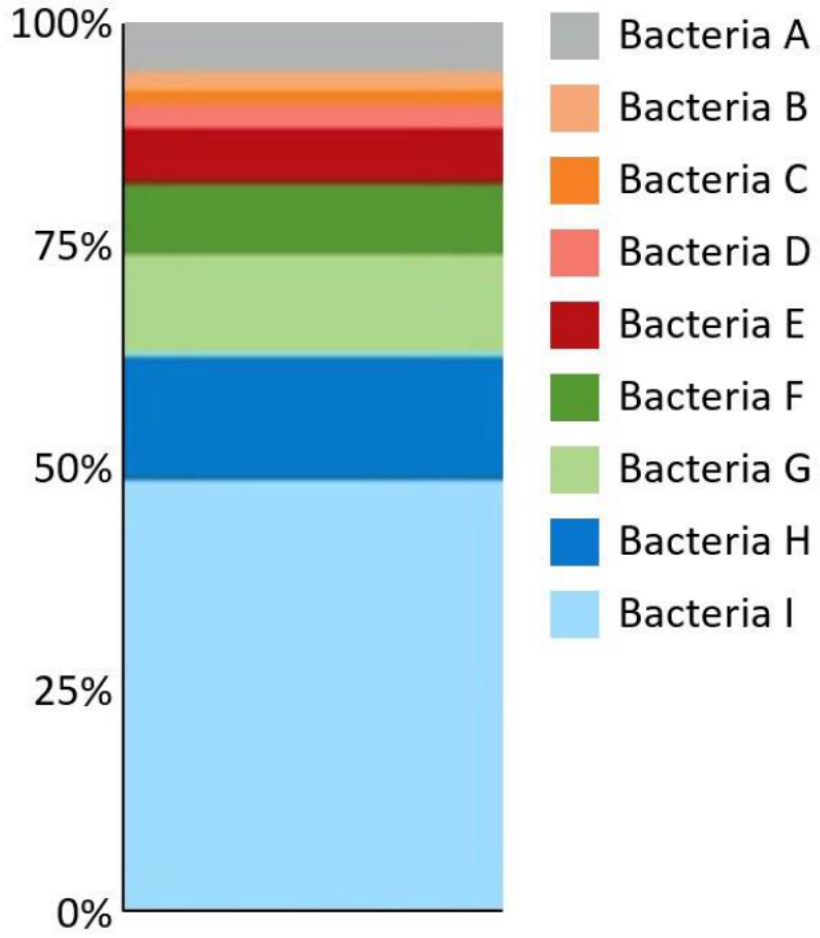
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #15

Figure #15



Is Bacteria C present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident — Somewhat confident — Very confident

(Place a mark on the scale above)

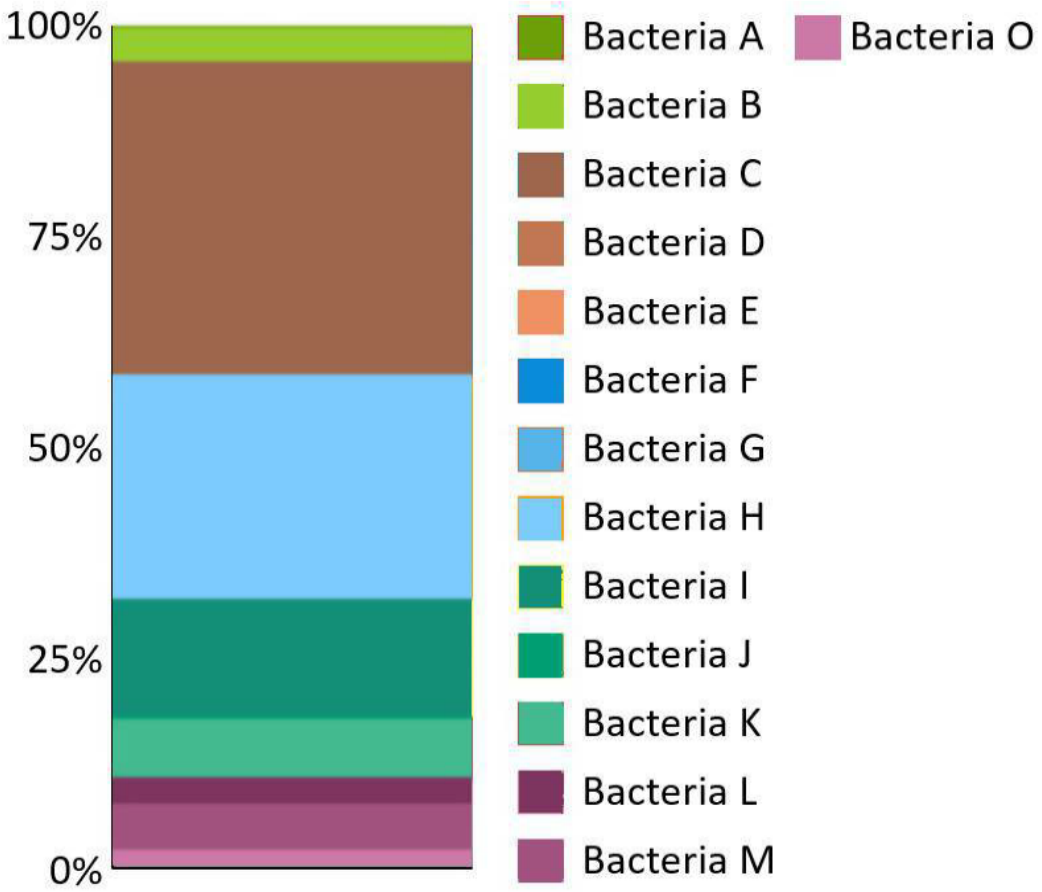
Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question:

Evaluation Question #16

Figure #16



Is Bacteria L present?

◯ Yes
◯ No

How confident are you in your previous response?

Not confident | Somewhat confident | Very confident

(Place a mark on the scale above)

Rate the figure's accessibility:
(Accessible = clear & easy to read; Not Accessible = confusing or difficult to read; Borderline = anything inbetween)

◯ Accessible
◯ Borderline
◯ Not Accessible

Please explain why you chose that accessibility rating in the previous question: