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Supplemental Information

Bacteriophages Isolated from Stunted Children

Can Regulate Gut Bacterial Communities

in an Age-Specific Manner

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SUPPLEMENTAL FIGURES

Figure S1. Related to all Figures. (a) Overview of experimental procedures. Schematic diagram of the experimental setup and procedures for one age group. These procedures were repeated in a second age group, leading to a total of 60 children surveyed. (b) Metagenomics workflow, with color-coded different types of analytical steps. * A cut-off value of e-value $<1e-5$ was used. When contigs showed similarity to different strains within one species, a common ancestor was used. ⌘ indicates that $>70\%$ coverage and $>90\%$ identity were used.

Figure S2. Related to Figures 1 and 3. Viral and bacterial abundances are similar across health status and age. Violin plots of whole community abundances of (a) Virus-Like Particles (VLPs), (b) Bacteria, and (c) the resulting Virus-to-bacteria ratio in fecal samples from 30 non-stunted and 30 stunted children aged 14-38 months. The enumeration of phage and bacterial cells was performed using epifluorescence microscopy (see Star Methods). N= 60 children total (15/age group/health status). The width of the violin plots reflects the frequency distribution of the data, dotted lines show the median and lower and upper quartiles.

Figure S3. Related to Figure 1. Shannon diversity (a, b) and richness (c, d) indices for bacterial phyla in the original samples show opposing patterns from those observed at the species level. Each index is compared among (a, c) the age according to the children's health status, and (b, d) the HAZ score according to the children's age group. In all cases, there is an interaction between the children's age and health status on Shannon diversity and richness indexes. In particular, we see that richness increases with HAZ, whereas Shannon decreases. Pools I-III: samples from the same age group were pooled randomly for sequencing (pool size was 3-5 samples).

Figure S4. Related to Figure 5. Changes in bacterial phyla Shannon evenness and richness in cross-infected cohorts among age groups. Shannon diversity (a) and richness (b) indices for bacterial phyla. Bacterial evenness decreased with age in stunted children (a), whereas the number of phyla either increased (stunted, green tones) or remained stable (non-stunted, red tones) with age (b).

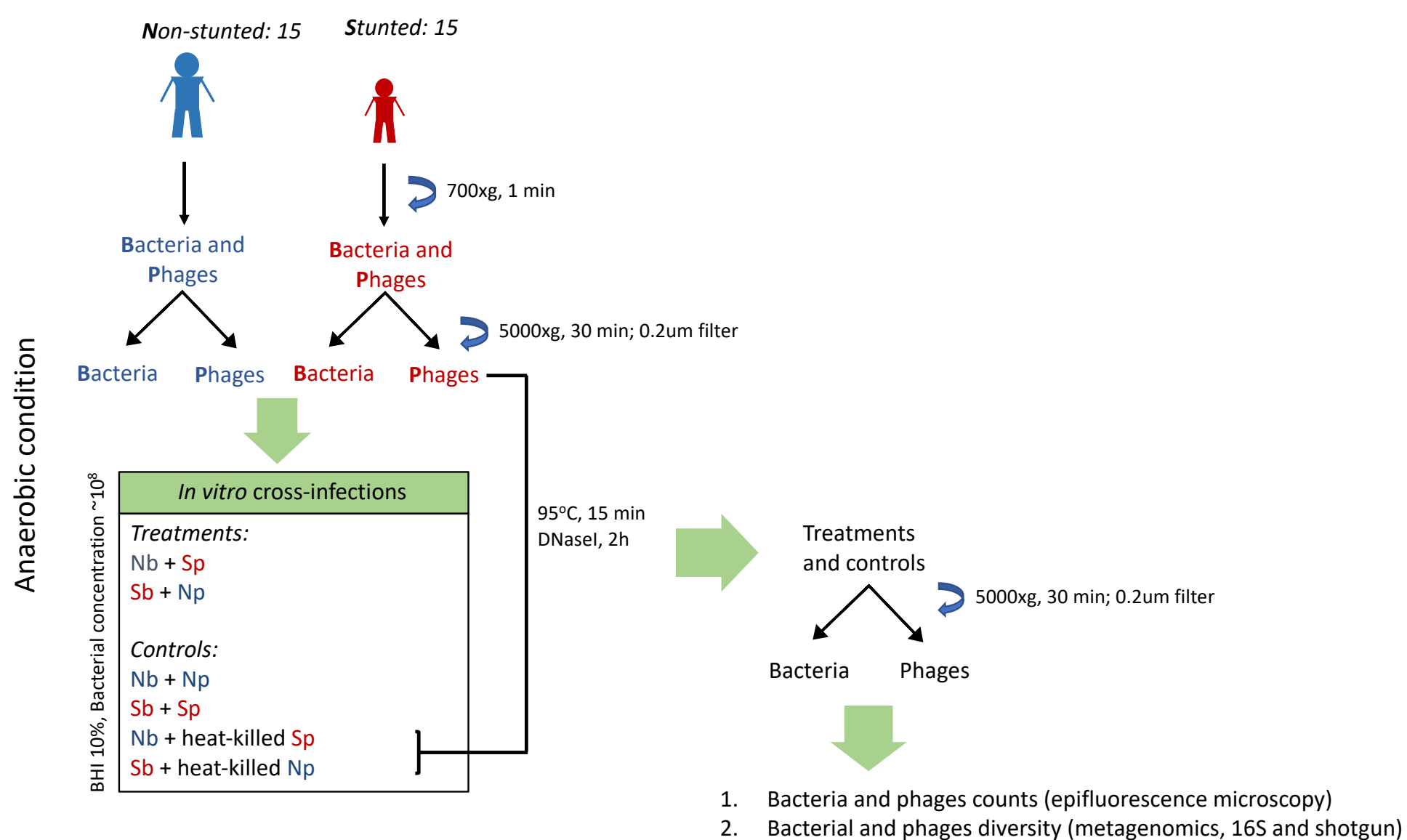
Figure S5. Related to Figure 5. Local contribution of samples to β -phage species diversity (LCBD) after cross-infections. Shown are the LCBD vs. treatment type for the younger (top) and the older (bottom) age groups, where cross-infection samples with non-stunted bacteria are shown in red and those with stunted bacteria are in green.

Figure S6. Related to Figure 6. Multiple Factor Analysis of all bacterial species, phage species, and children's metadata in younger children. Quantitative variables: milk feed, age, sex, HAZ, WAZ, and WHZ; qualitative variables: health status, treatment type, and treatment factor. Shown in the first panel is the ordination of qualitative and in the second and third panel the contribution of the quantitative variables to dimensions 1 and 2, respectively, where the dashed red line separates the variables that contribute the most (higher than average contribution of all variables) to the MFA. The Hellinger transformation of the bacteria species, phage species, and phage replication cycle data are presented here.

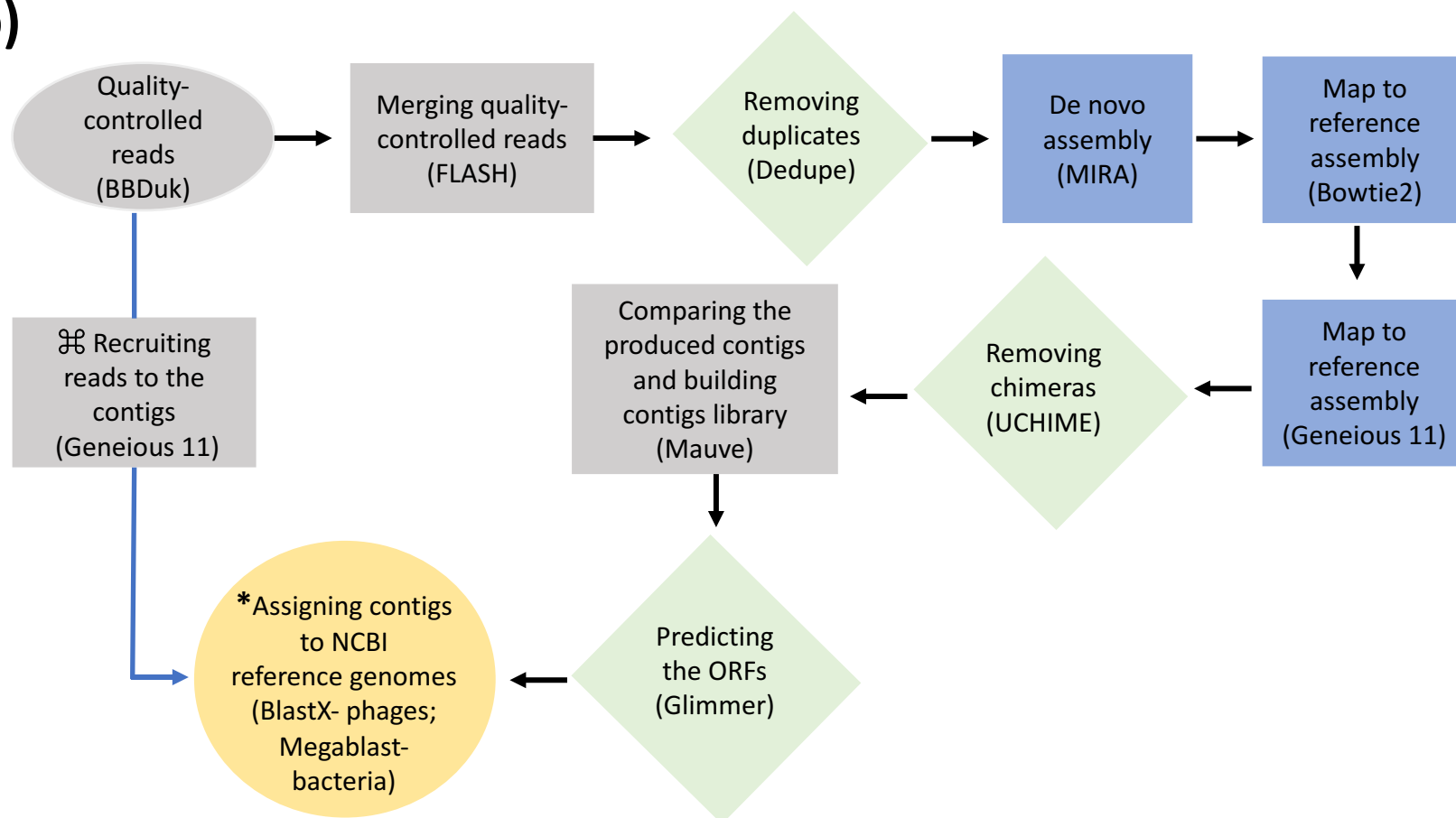
Figure S7. Related to Figure 6. Multiple Factor Analysis of all bacterial species, phage species, and children's metadata in older children. Quantitative variables: milk feed, age, sex, HAZ, WAZ, and WHZ; qualitative variables: health status, treatment type, and treatment factor. Shown in the first panel is the ordination of qualitative and in the second and third panel the contribution of the quantitative variables to dimensions 1 and 2, respectively, where the dashed red line separates the variables that contribute the most (higher than average contribution of all variables) to the MFA. The Hellinger transformation of the bacteria species, phage species, and phage replication cycle data are presented here.

Figure S8. Related to Figure 7. Phage communities alter functional traits of bacterial community in an age-specific manner. (a) MFA of the dominant functional traits and children metadata for both age groups. Dominant functional traits shown were selected using a PCA and the contribution circle from the `cleanplot.pca()` function. MFA ordination of the qualitative variables (health status, treatment type, and treatment factor). The Hellinger transformation of the bacterial, phage, and replication cycle data are presented here. (b) Relationship between the local contribution of samples to beta diversity of the functional traits (LCBD) after cross-infections for bacteria from (top panels) and phages (bottom panels).

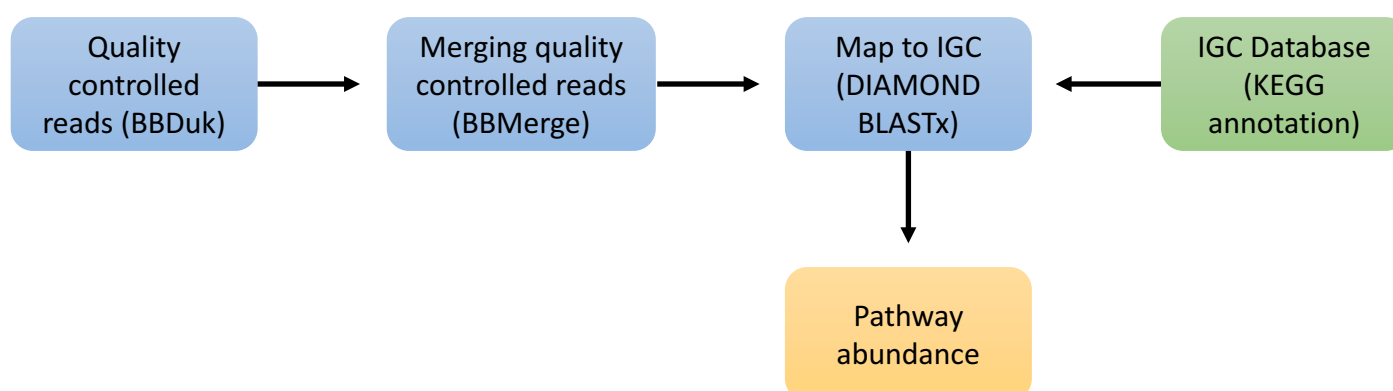
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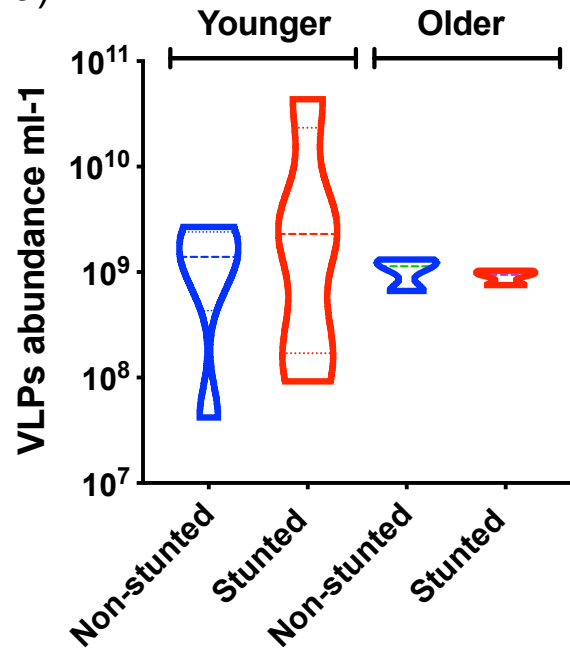
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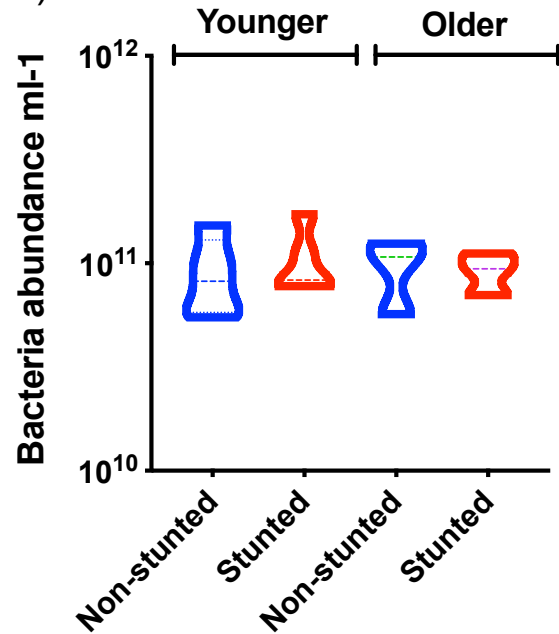
c)



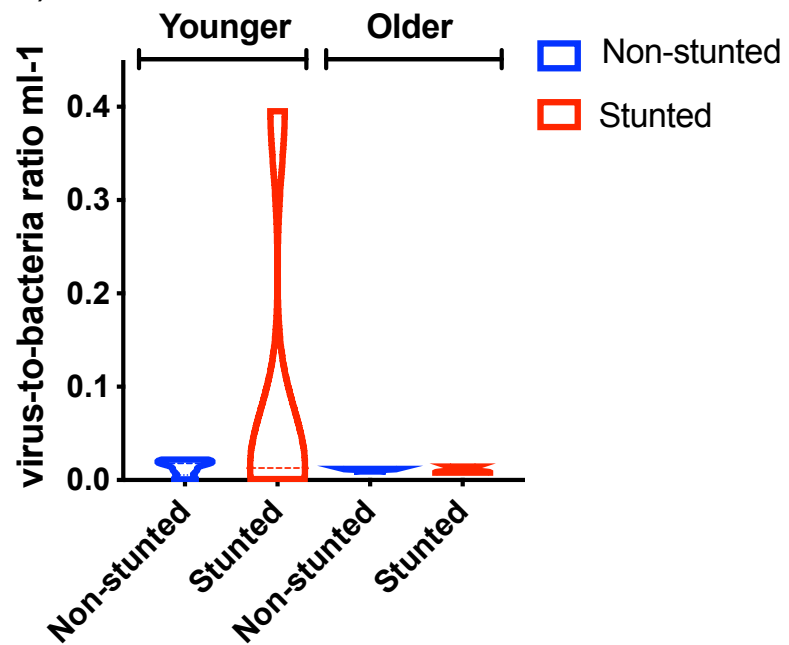
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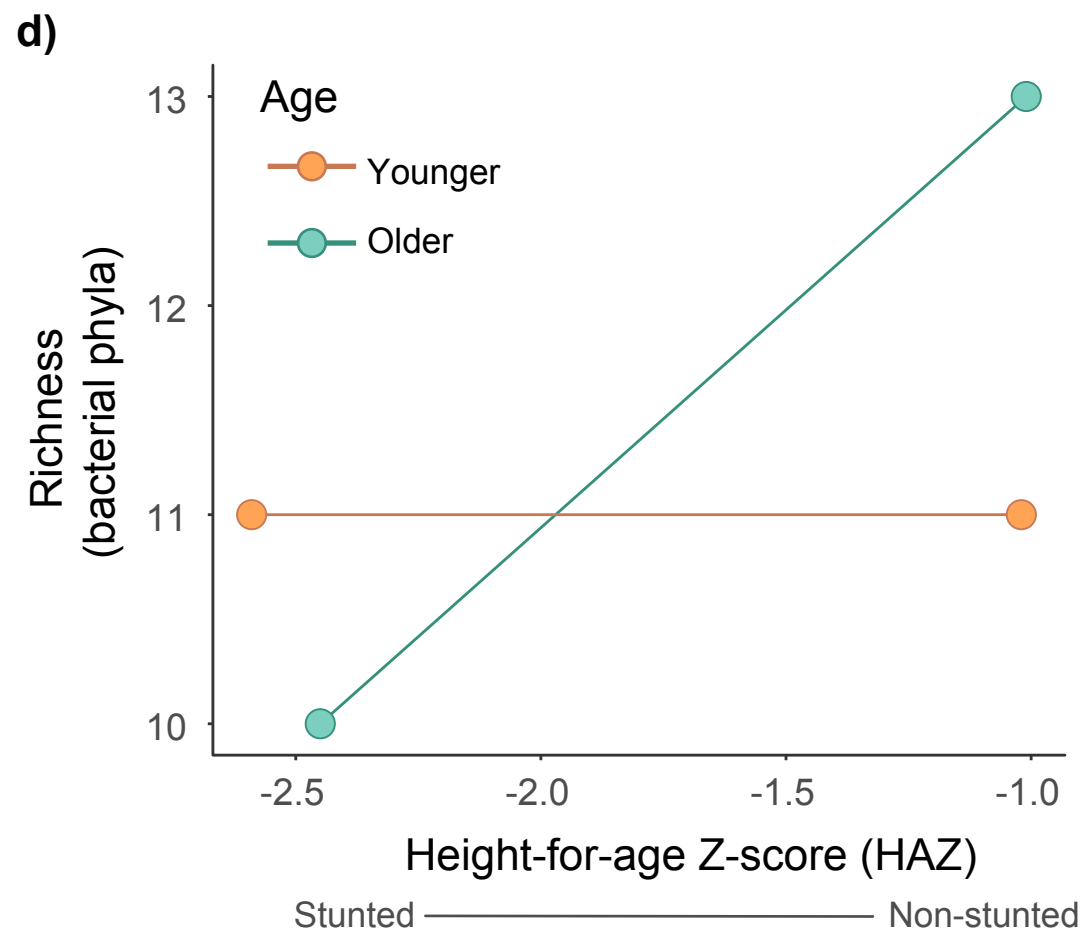
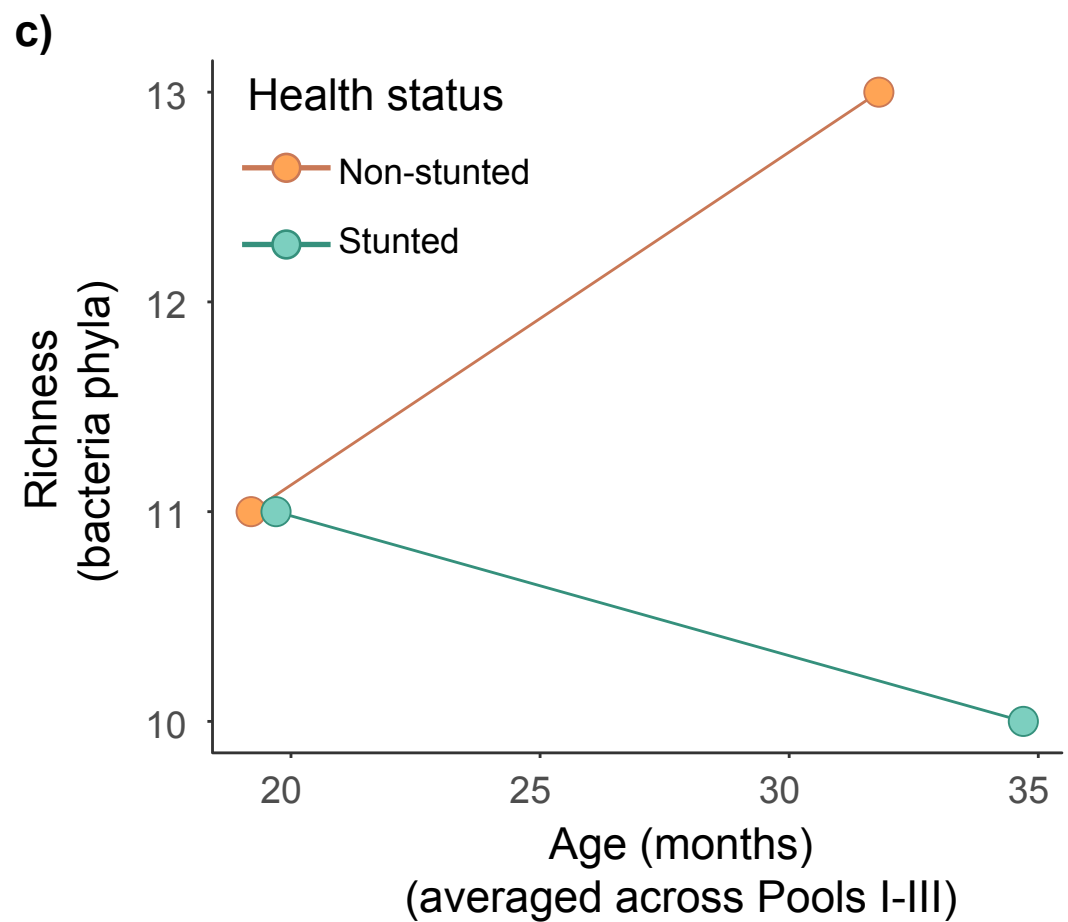
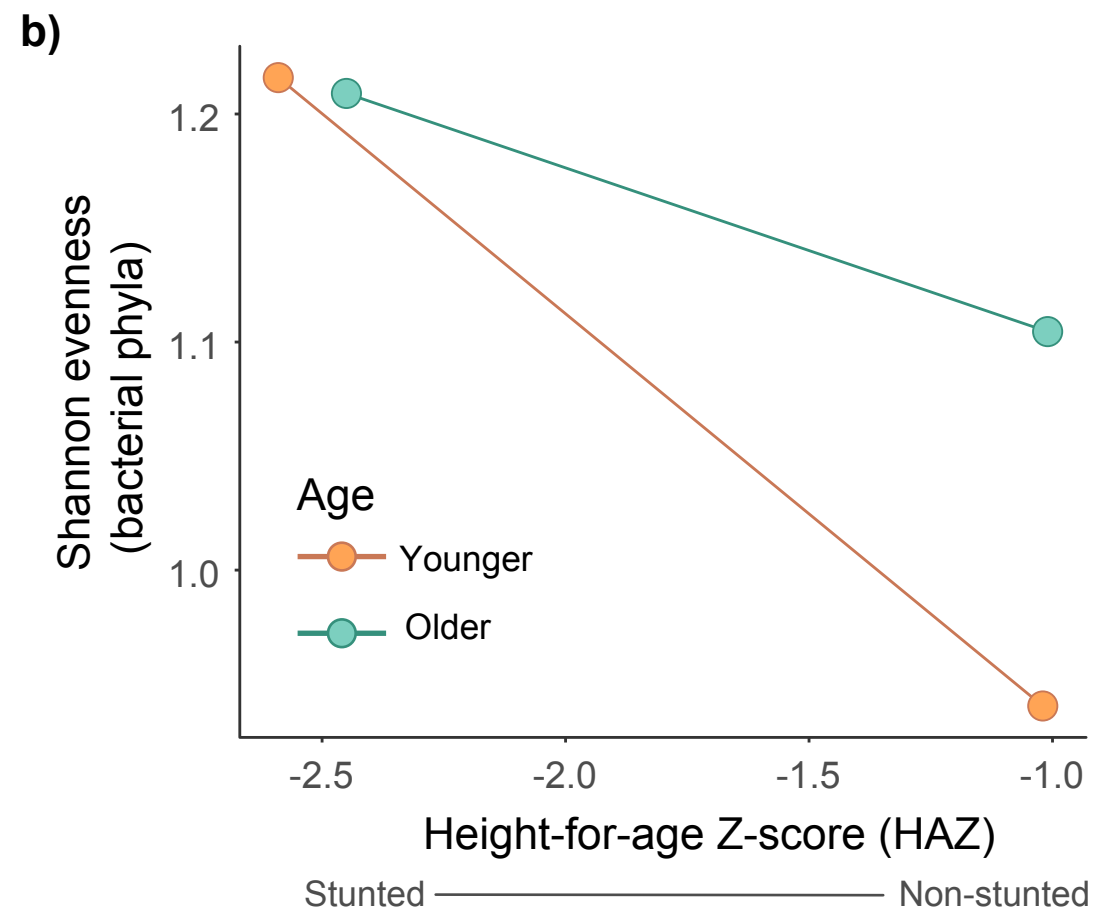
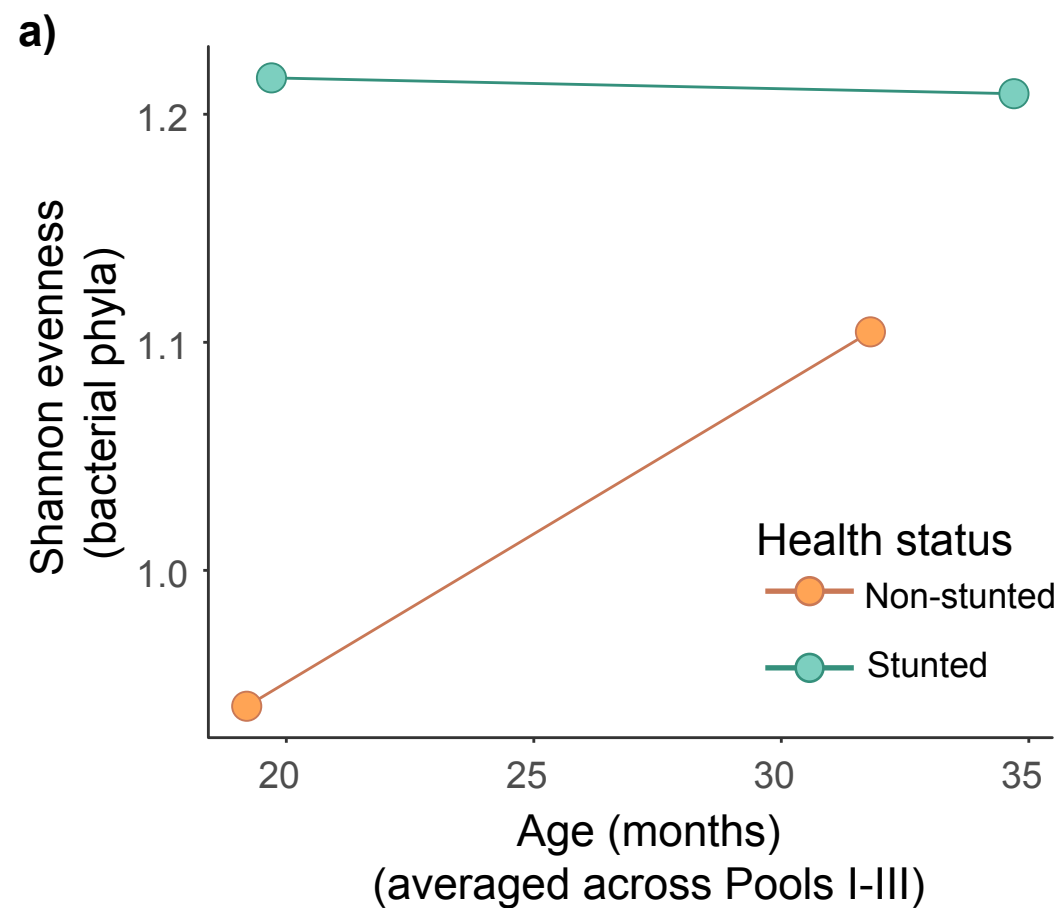


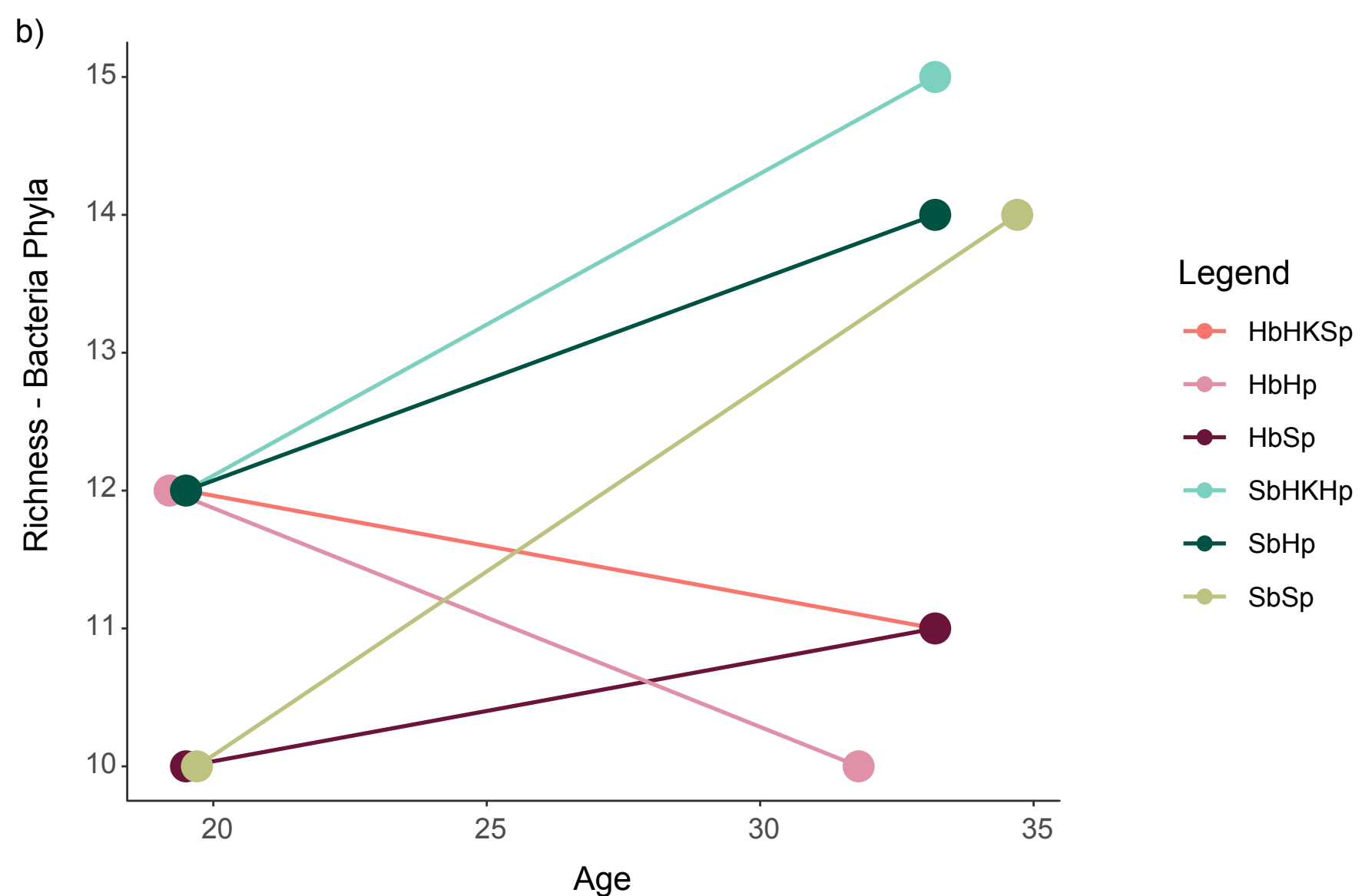
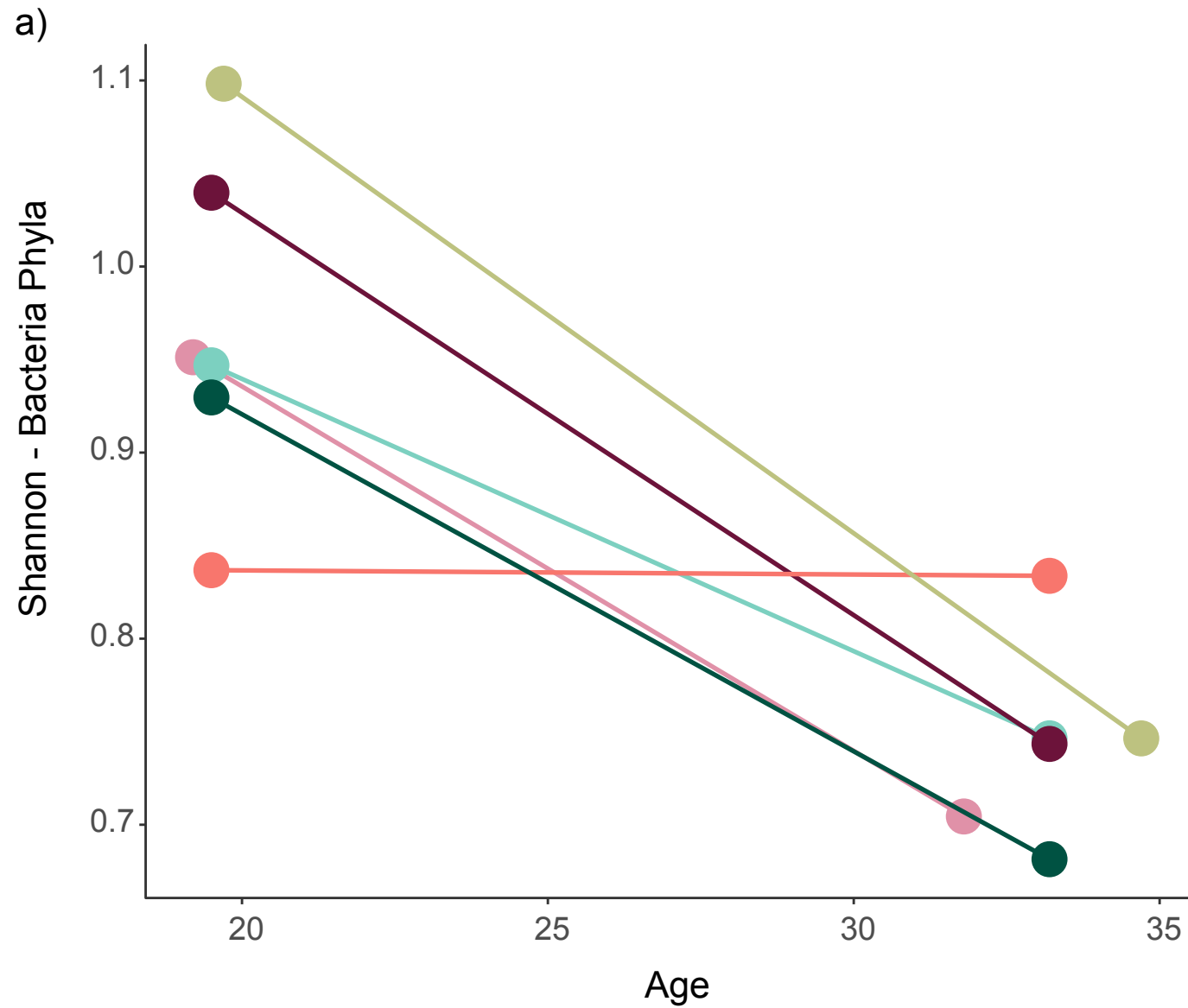
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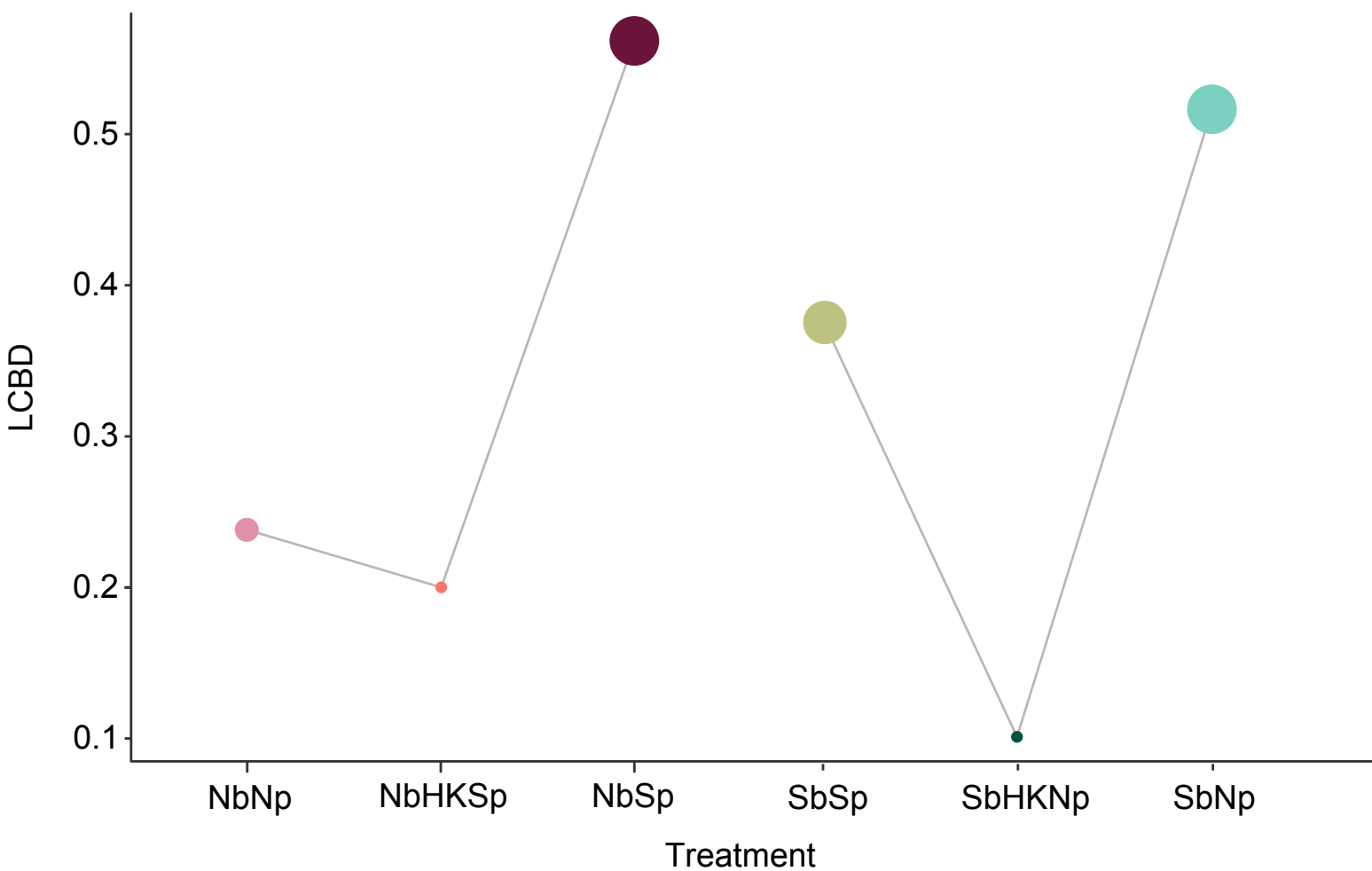
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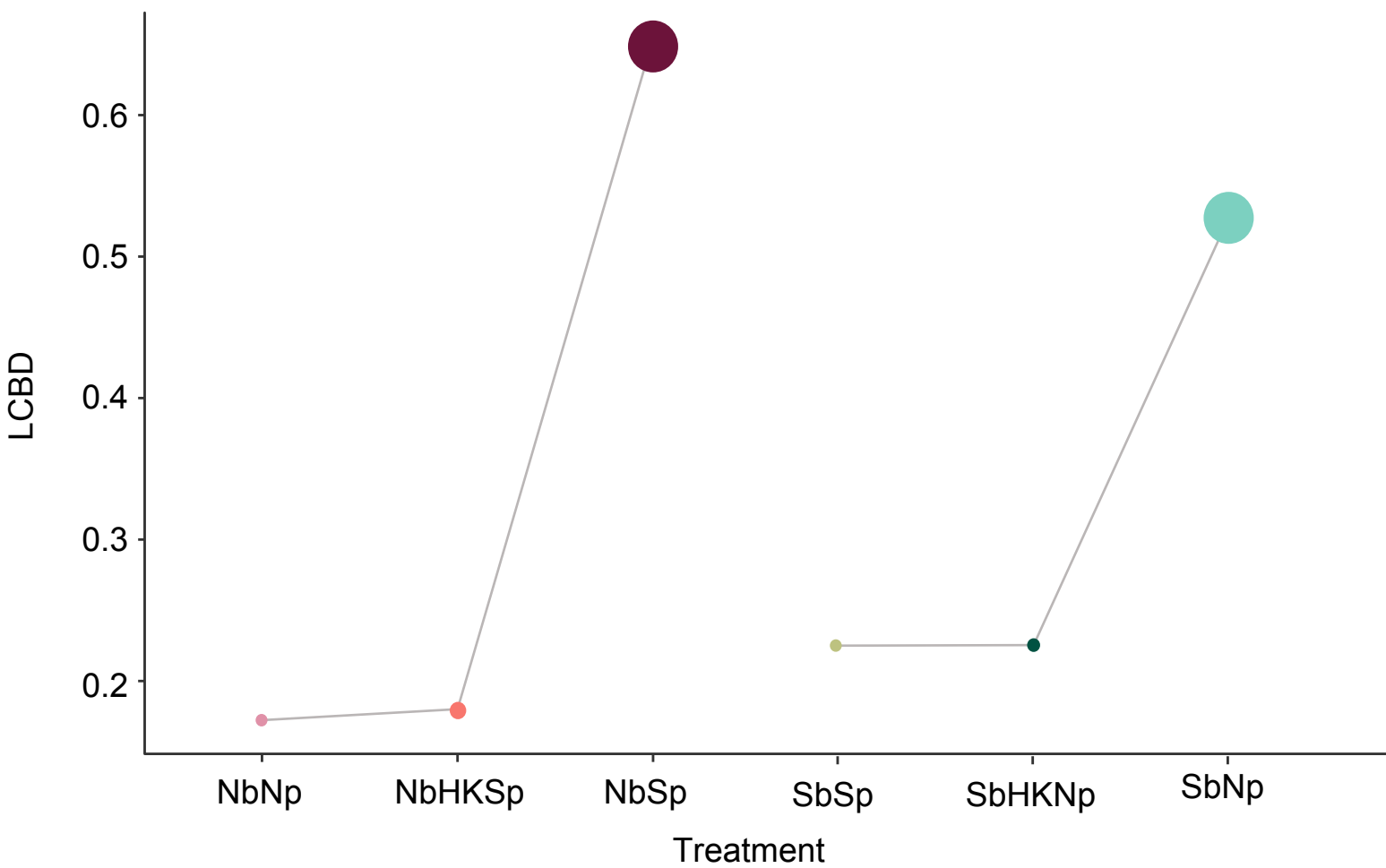




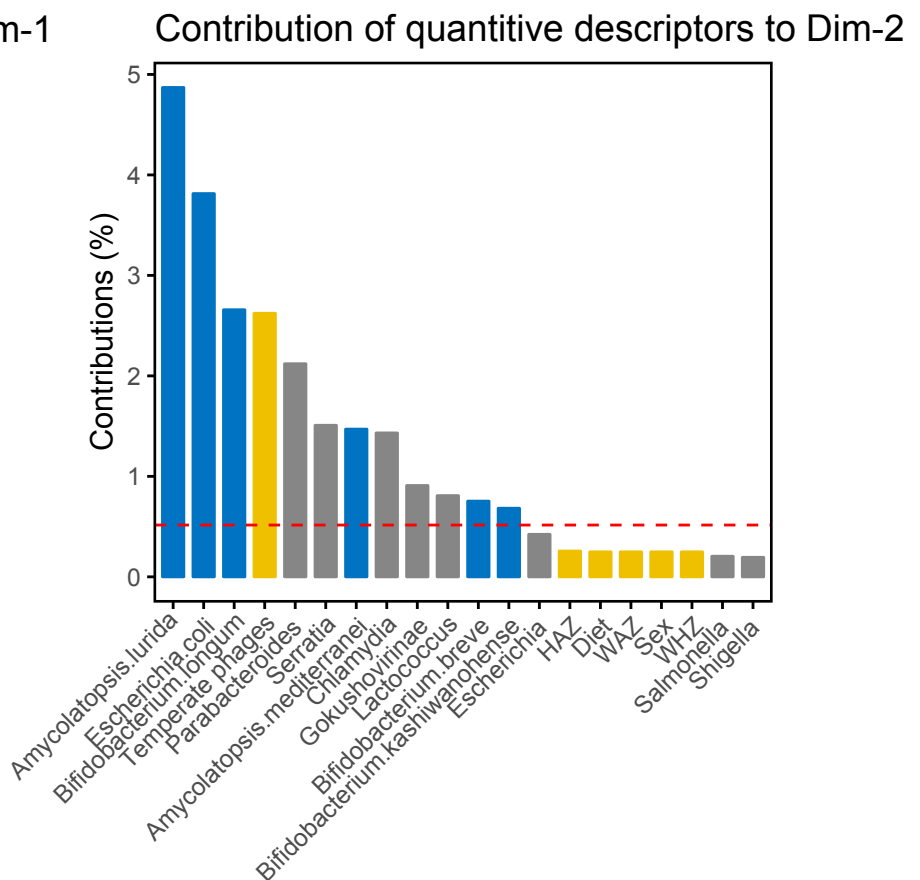
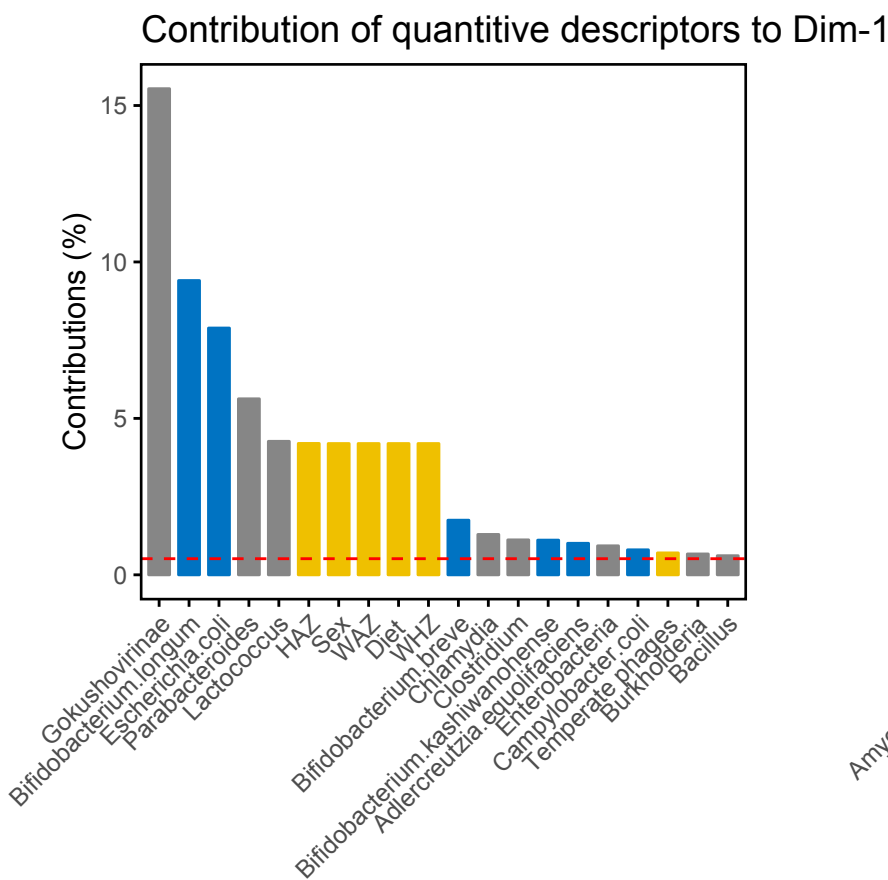
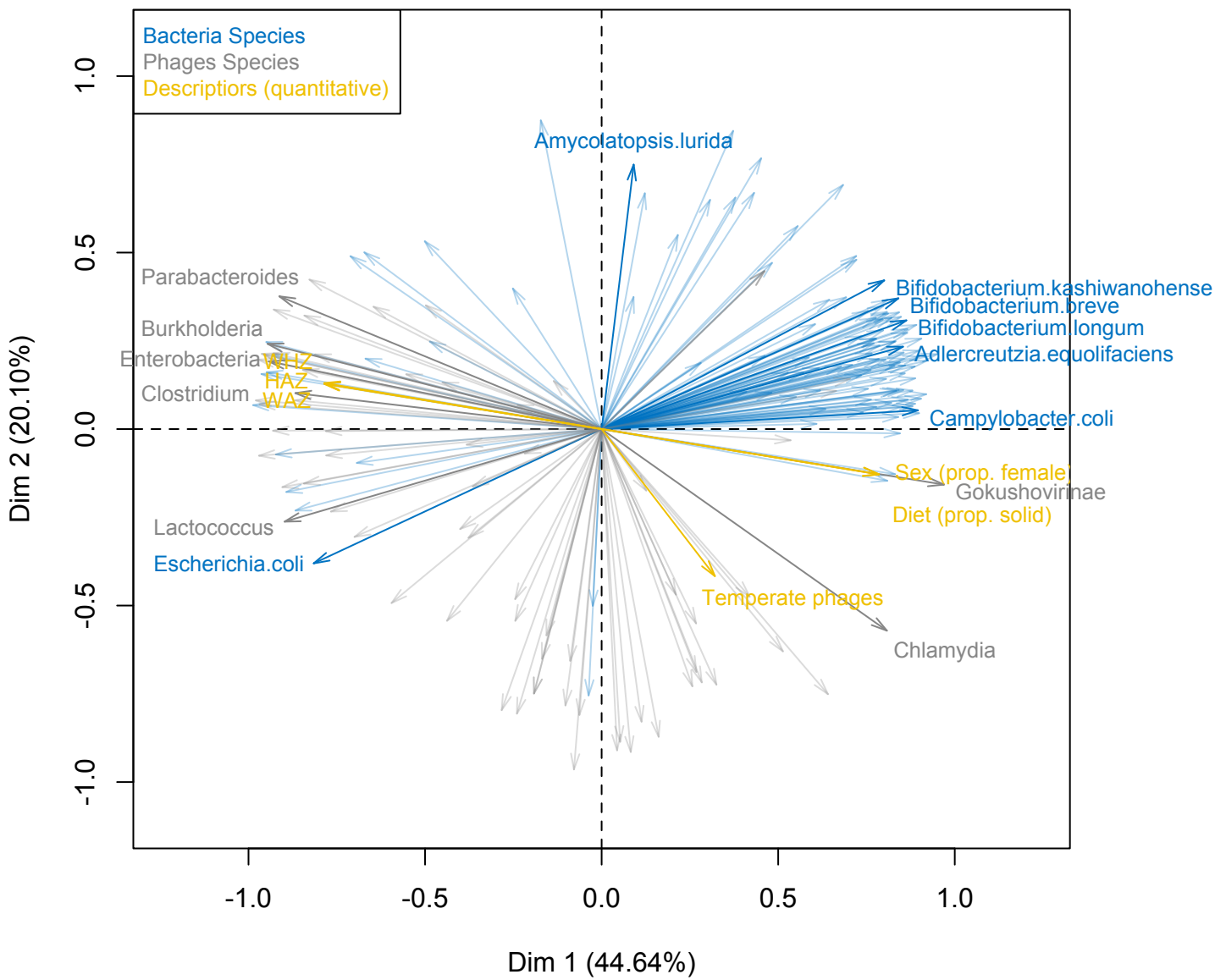
Younger: 14-23 mo



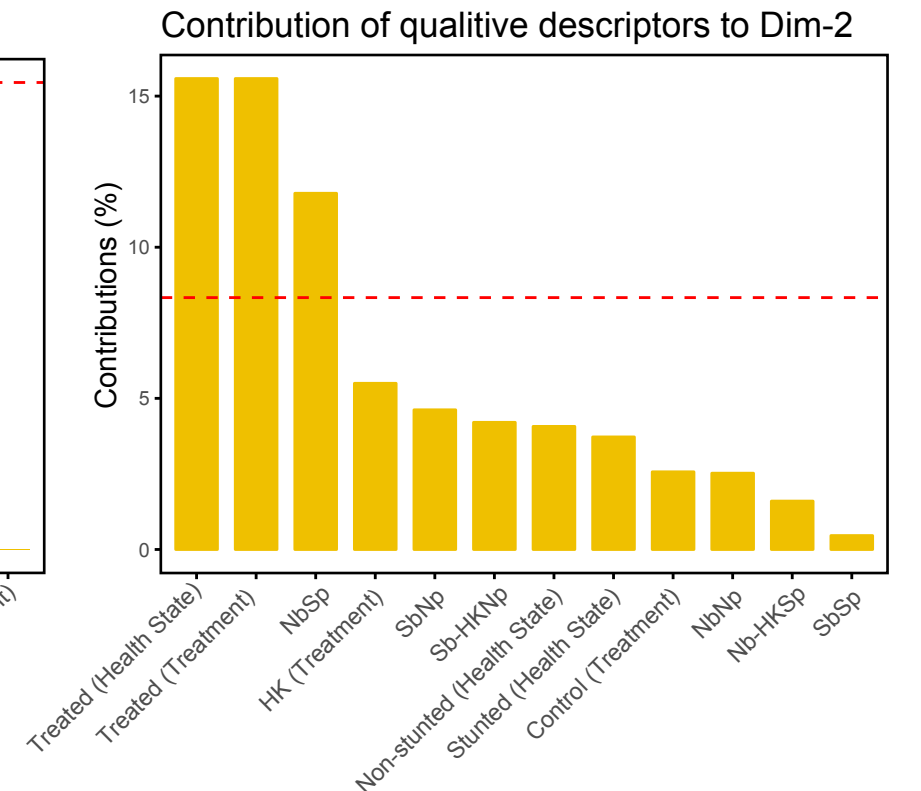
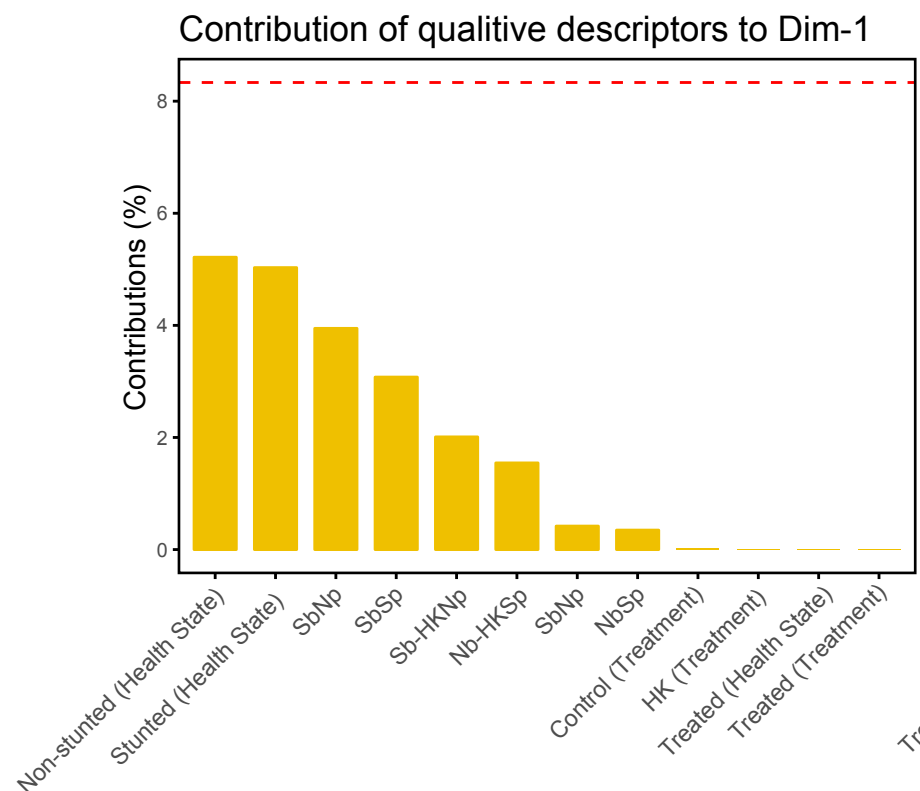
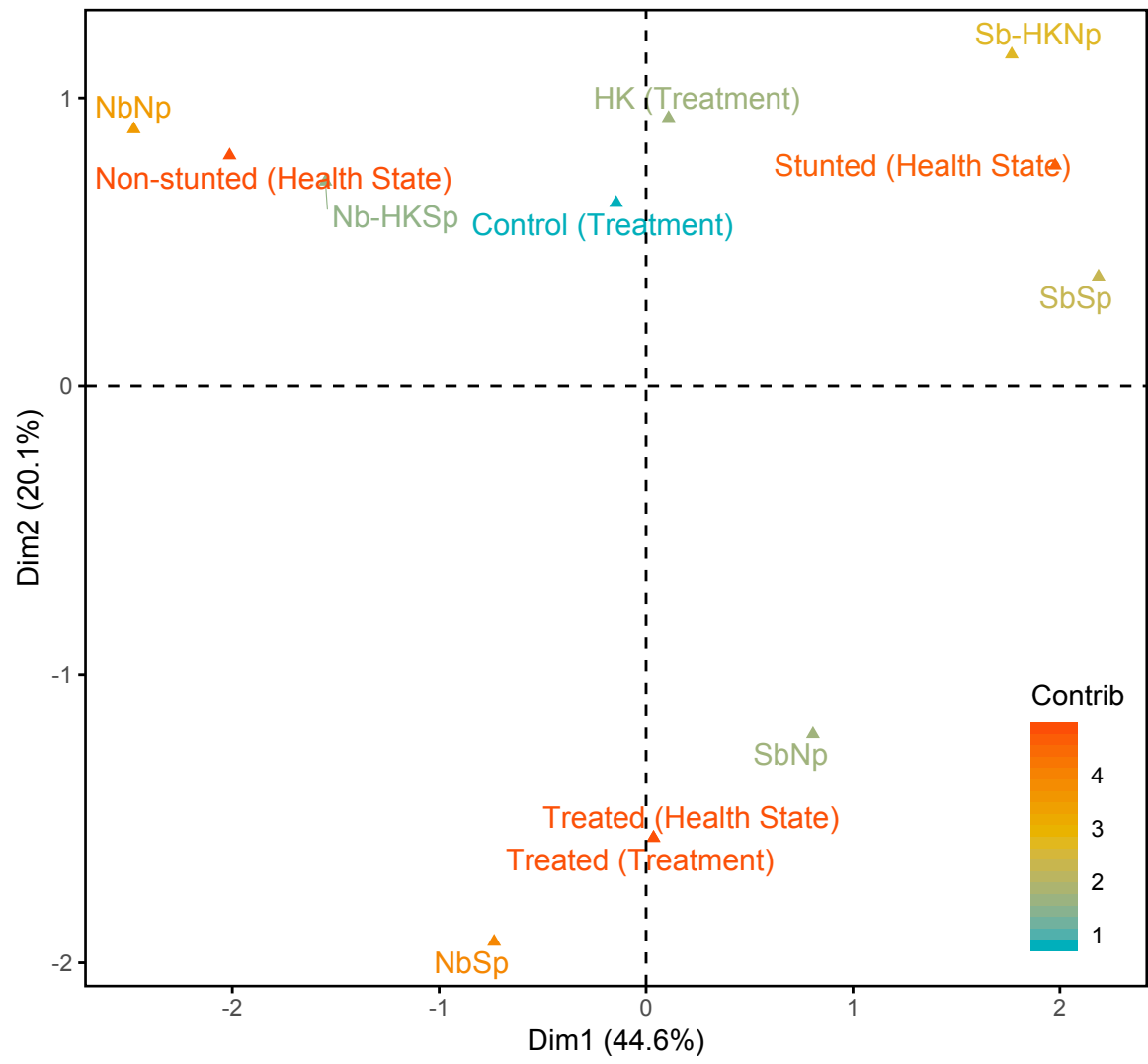
Older: 23-38 mo



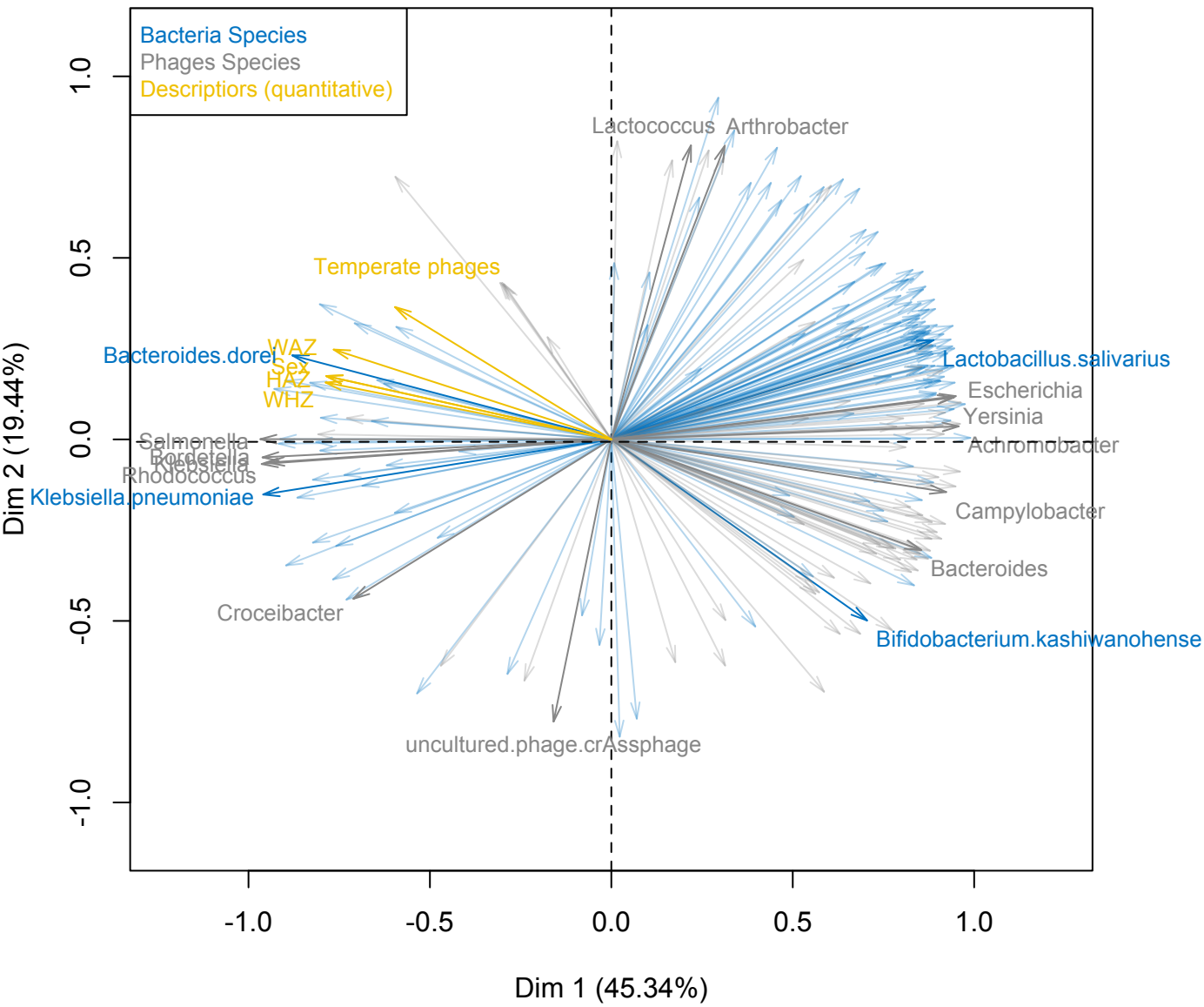
a) Quantitative variables



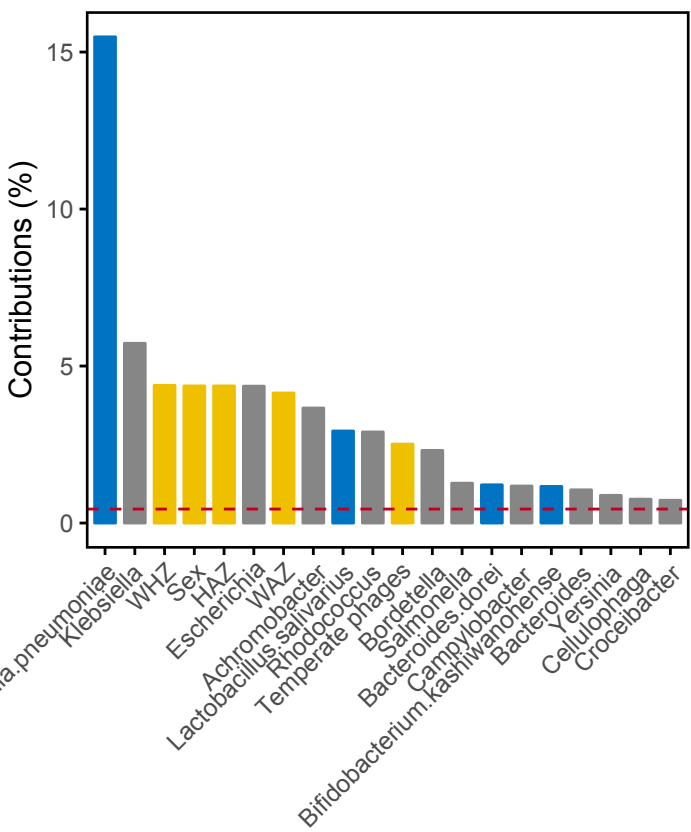
b) Qualitative descriptors



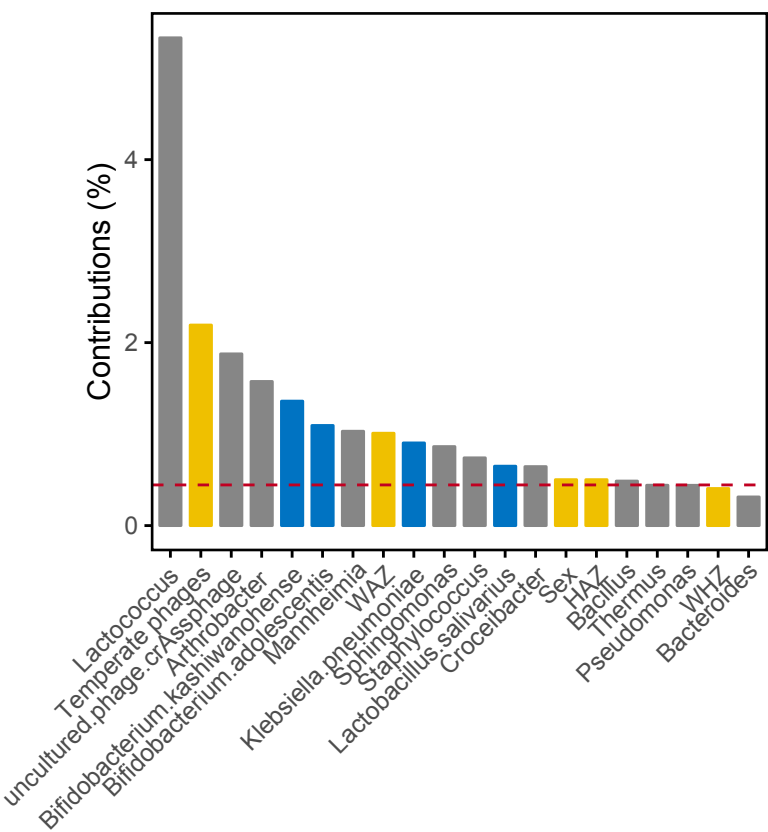
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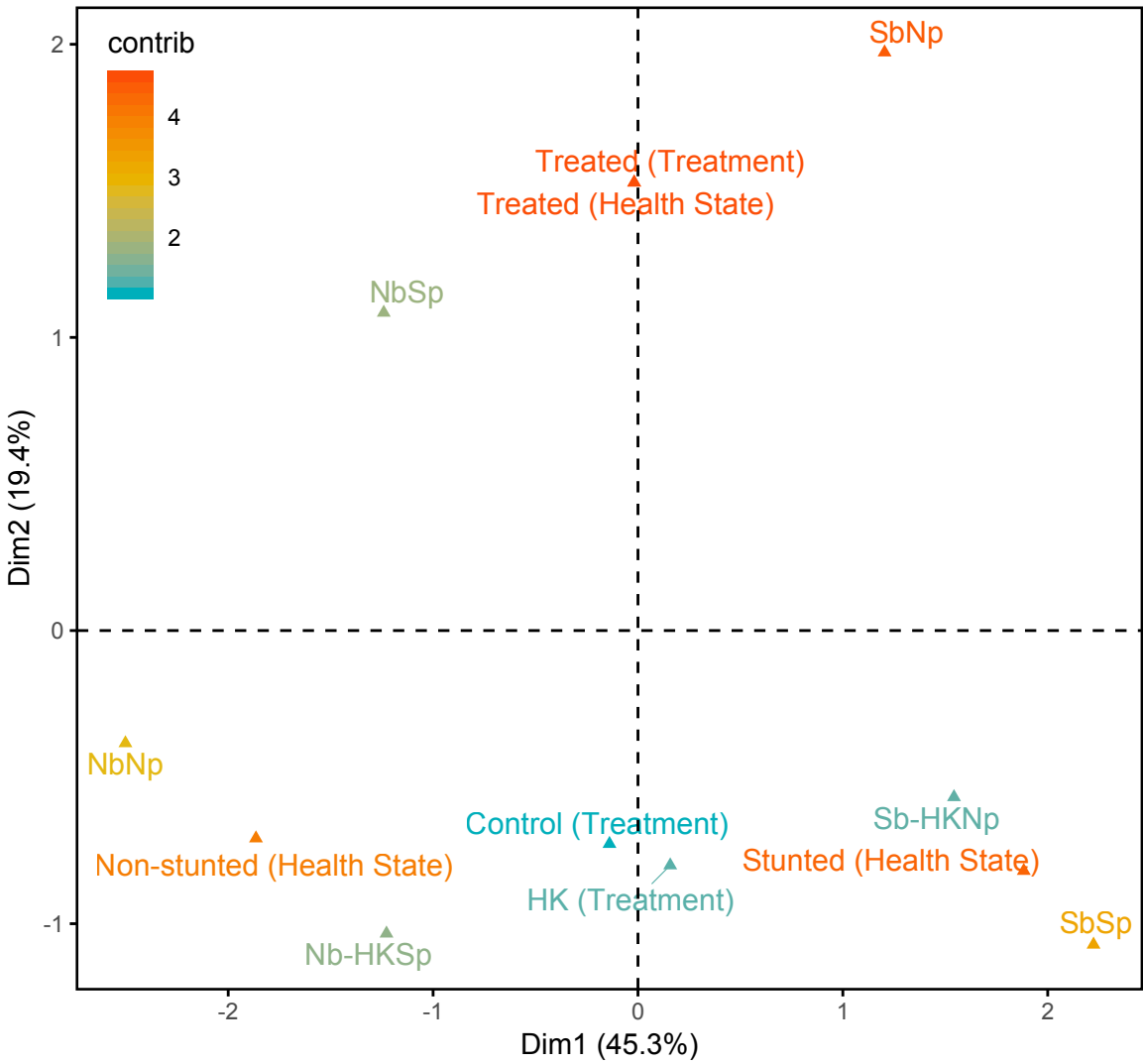
Contribution of quantitive variables to Dim-1



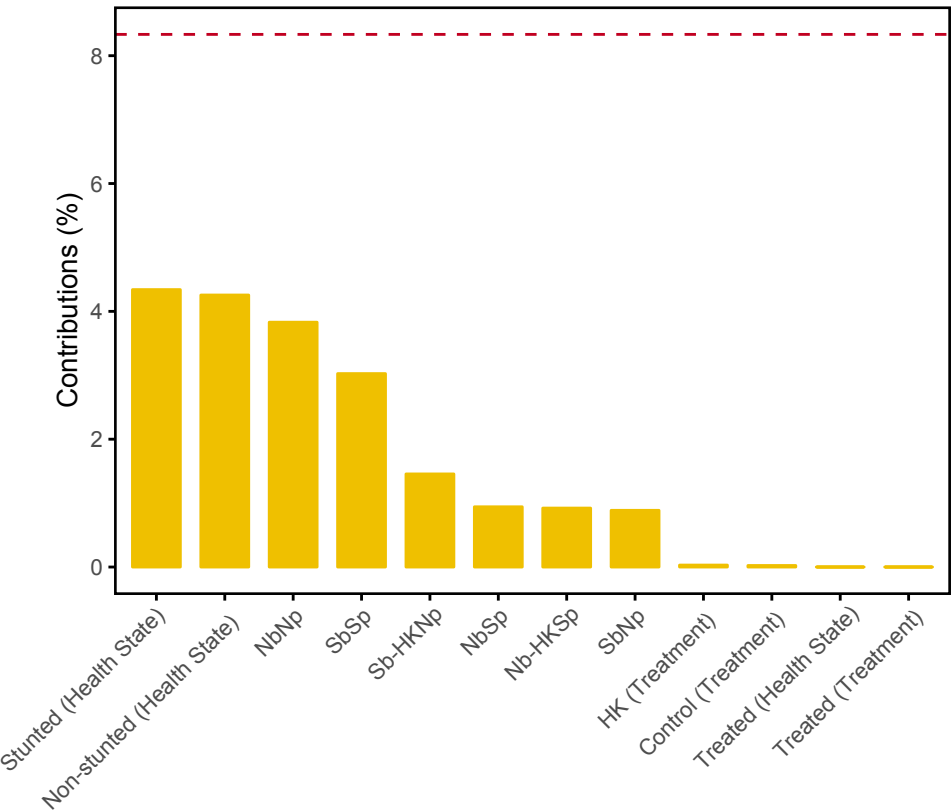
Contribution of quantitive variables to Dim-2



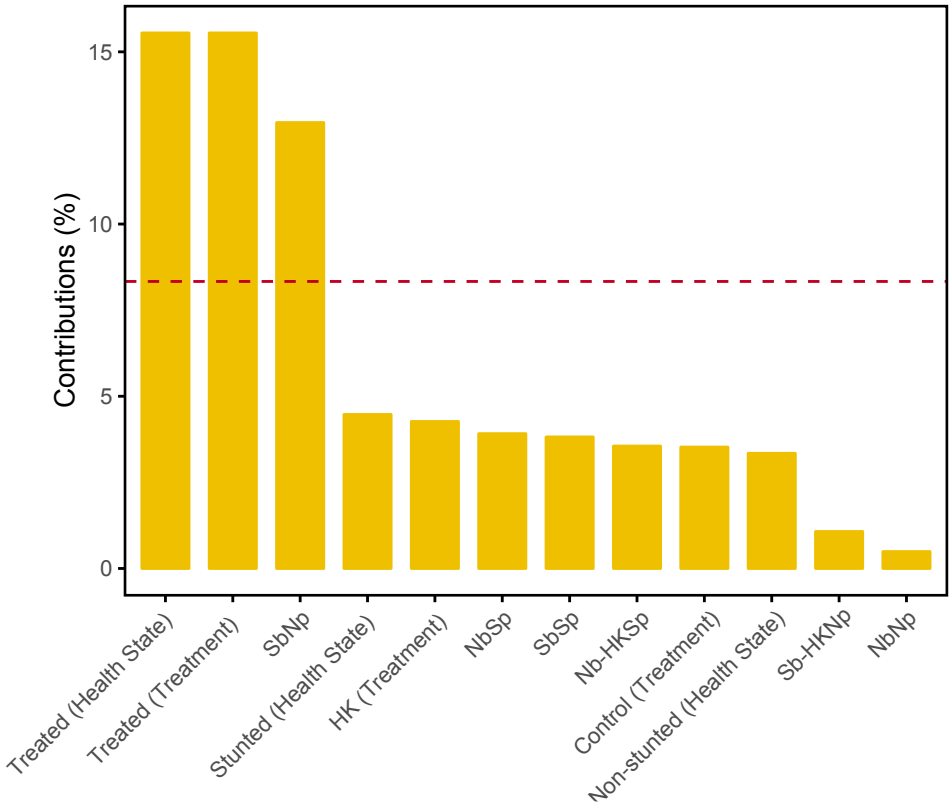
b) Qualitative descriptors



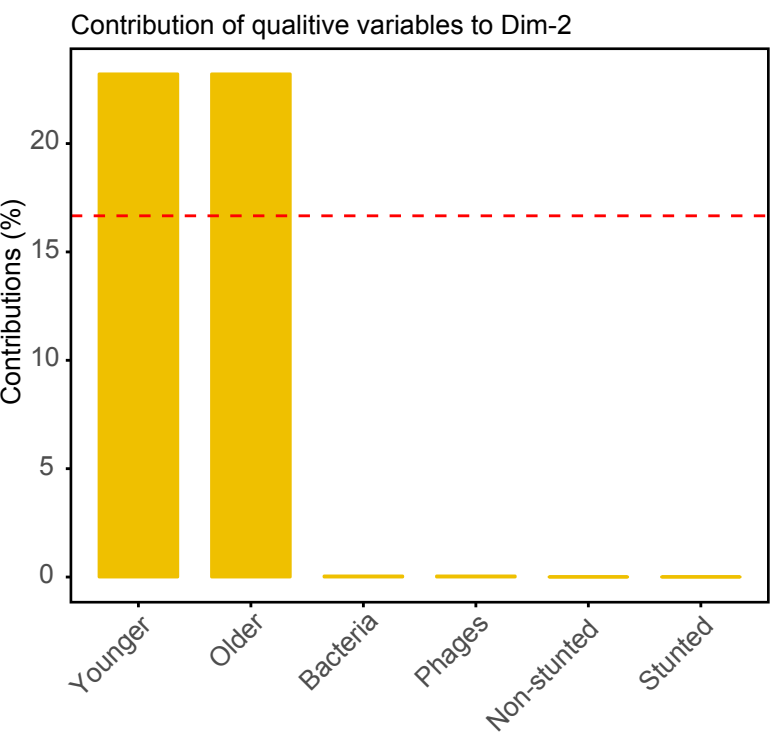
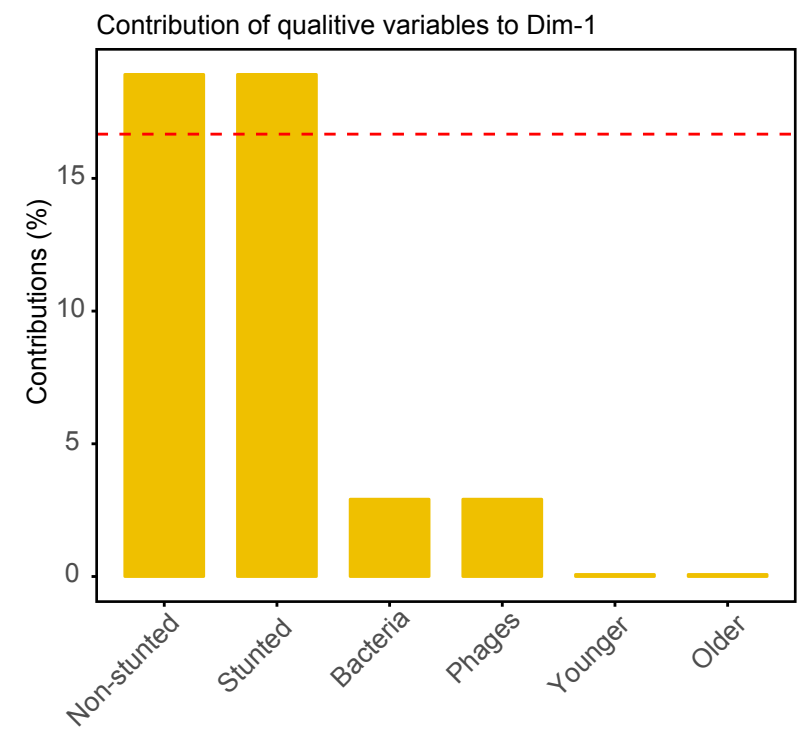
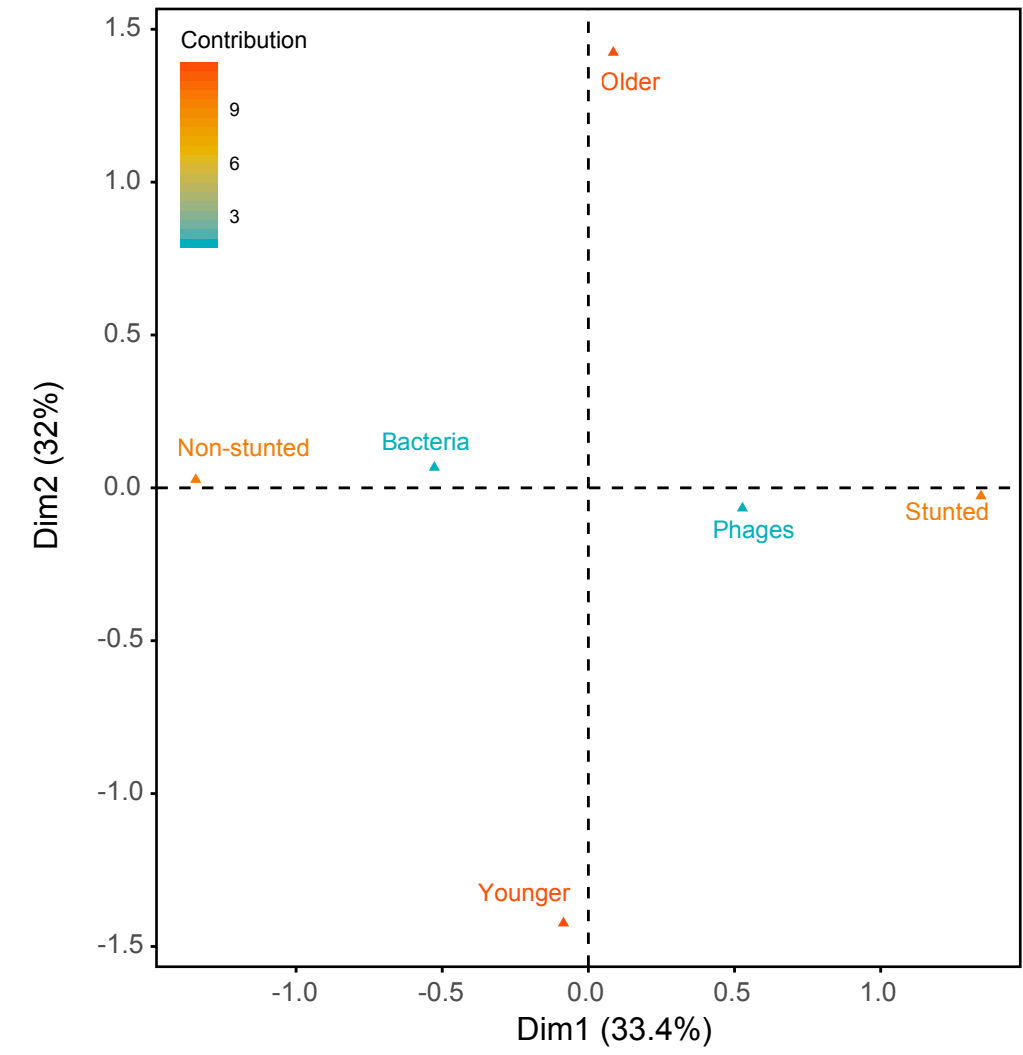
Contribution of qualitative variables to Dim-1



Contribution of qualitative variables to Dim-2



a)



b)

