Bow River Microbiome – Beta Diversity

PCoA with Bray Curtis distance matrix on rarefied data (sequencing depth of 2180).

Coloured by Site

Diagram

Description automatically generated

**Figure 1:** Principal coordinate analysis (PCoA) of dissimilarity of bacterial beta diversity between sampling sites using Bray-Curtis distance matrix. Data was log (1 + x) transformed prior to ordination.

Many of the 95% confidence intervals are overlapping (variation in the data is overlapping) but there were significant pairwise differences in the centroid of the beta diversity cloud across all sites except PCR1 – PCR3 (ACWA streams) and Cochrane – Sunalta using a PERMANOVA.

There was also significantly different beta dispersion between sites which may indicate that there could be false positives in the significant adonis results.

Coloured by sample Family:

A picture containing diagram

Description automatically generated

**Figure 2:** Principal coordinate analysis (PCoA) of dissimilarity of bacterial beta diversity between sample taxonomic family using Bray-Curtis distance matrix. Data was log (1 + x) transformed prior to ordination.

There were significant bacterial beta diversity differences between all sample families using PERMANOVA.

Coloured by sampling Month:

Chart

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**Figure 3:** Principal coordinate analysis (PCoA) of dissimilarity of bacterial beta diversity between sampling months (July versus September) using Bray-Curtis distance matrix. Data was log (1 + x) transformed prior to ordination.

There was a significant difference in beta diversity in samples collected in July versus September 2022. It looks like the samples in July group into three clusters which may be indicative of site or family of sample.

There was also significant beta dispersion between groups.

The U-shaped graphs indicate that an NMDS should be used instead. Graphs to follow:

NMDS:

Chart

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There are 2 samples that are extreme outliers causing the graph to look weird. One is CUSHBR\_1\_Ad\_Caddis\_MB\_2 and the other is POLFLT\_1\_Ad\_Caddis\_1 (I am still missing 8 of the pmf caddis samples so this is probably why).

With these two samples removed looks much better:

Chart

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Figure 5 Non-metric multidimentional scaling of larval and adult macroinvertebrate and spider microbiome composition across sampling sites. Each point represents a single microbiome sample. 2D stress D 0.17.

Many ellipses are overlapping but PERMANOVA indicates that there are significant differences between all sites except PCR1 - PCR3 and Cochrane – Sunalta (PERMANOVA, F2,8 = 3.66, p = 0.001). There is also significant beta dispersion between groups too though, so these results need to be taken with a grain of salt (ANOVA, F = 83.2, P < 0.001).

Because of this, when reporting results the ordination should be included and the significant PERMANOVA and PERMDIST need to be reported. The significant difference in centroids could just be because the variance is not homogenous across groups (high variability).

Chart, bubble chart

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Figure 6. Non-metric multidimentional scaling of larval and adult macroinvertebrate and spider microbiome composition across sample taxa. Each point represents a single microbiome sample. 2D stress D 0.17.

Significant differences in beta diversity across all sample families.