

Additional file 1: Supplementary figures

Microbial core communities in activated sludge plants are strongly affected by immigration and geography

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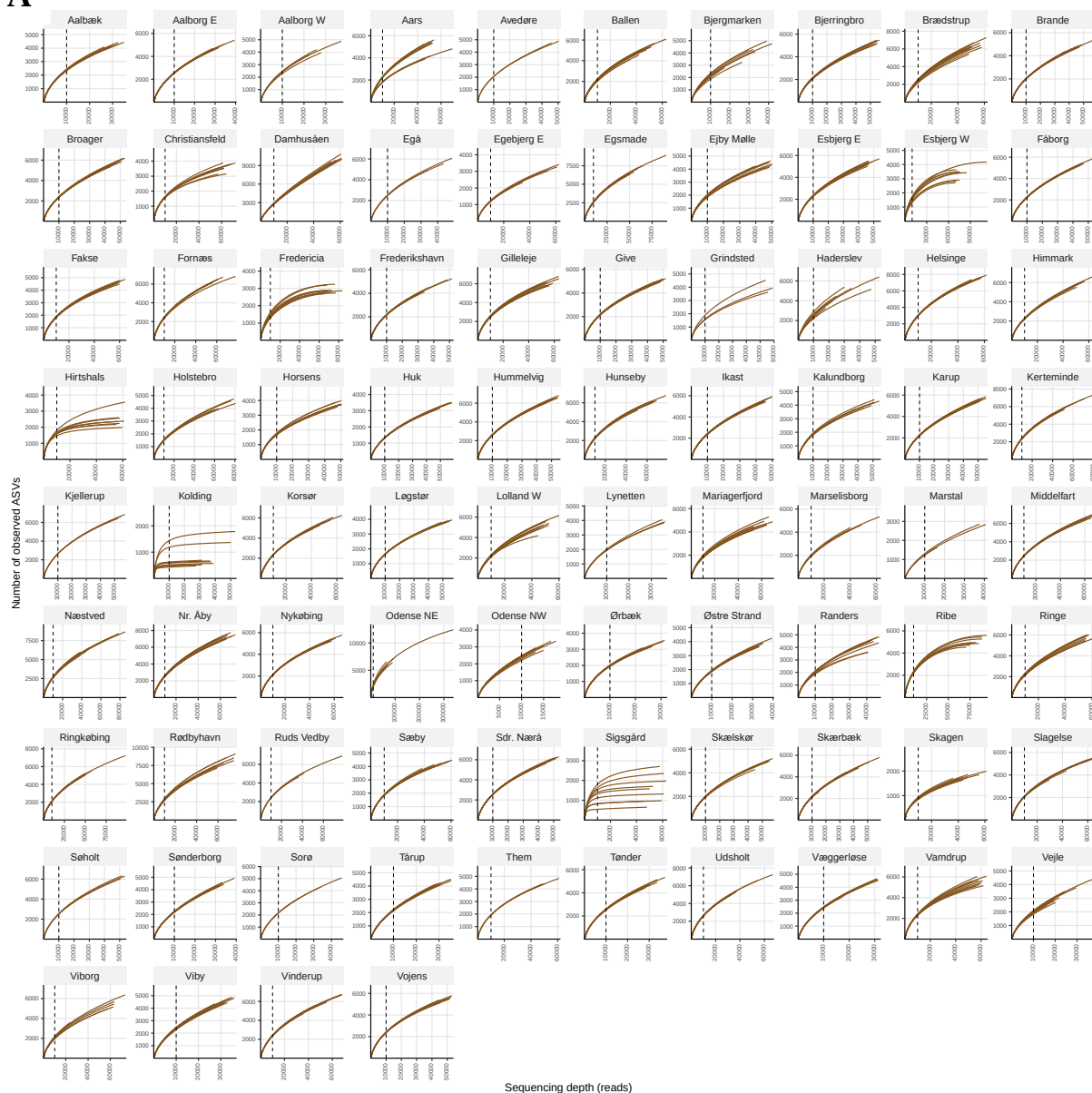
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Figure S1. Sampling campaign. Dates of sample collection grouped by plant and colored by sample content, either activated sludge (AS) or influent wastewater (IWW). No sample dates for Brødstrup or Ringkøbing were reported and therefore not plotted, however sampled within the sampling period of September-December 2019 with 10 samples of both AS and IWW for each plant.

A



B

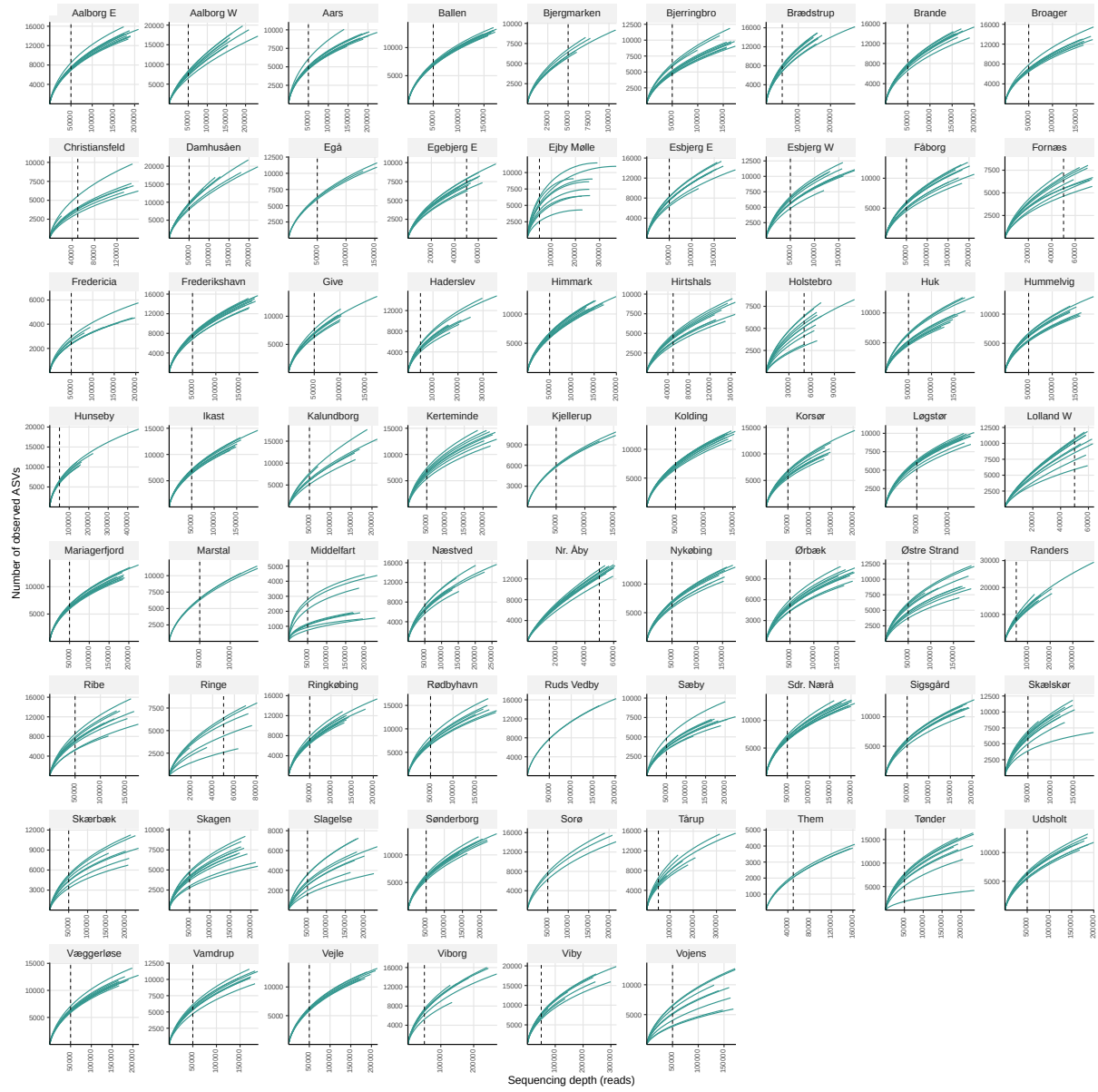


Figure S2. Rarefaction curves for activated sludge (A) and influent wastewater (B). A vertical dashed line is added at 10,000 and 50,000 reads for activated sludge and influent wastewater plots, respectively.

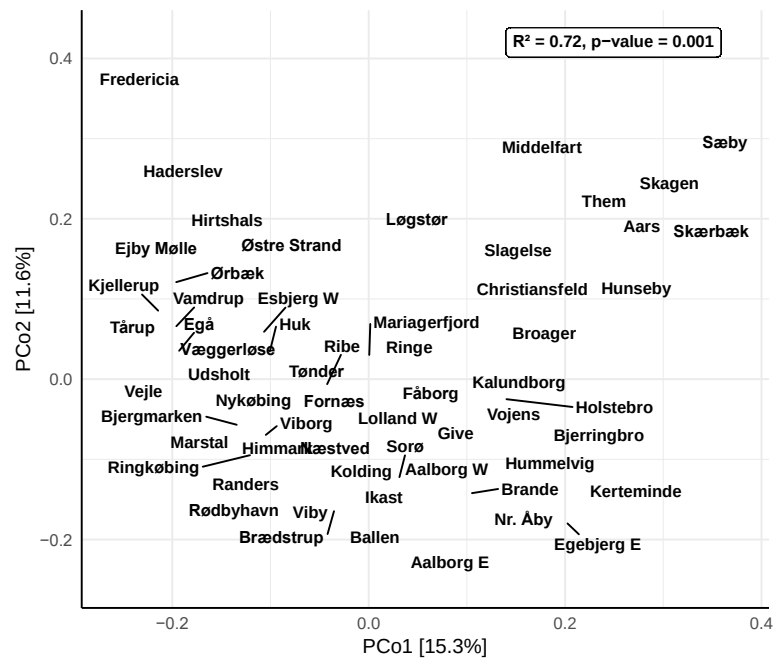
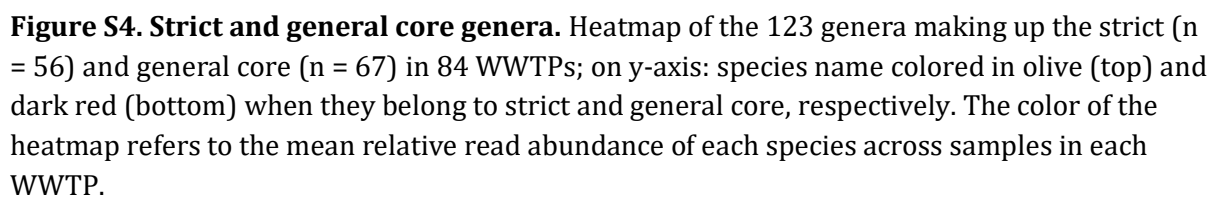


Figure S3. Community beta diversity in IWW. Beta-diversity in IWW samples of 69 plants visualized as PCoA plot based on Bray-Curtis dissimilarity matrix. The explained variance (R^2) refers to differences of communities based on WWTP, each represented by a different color.



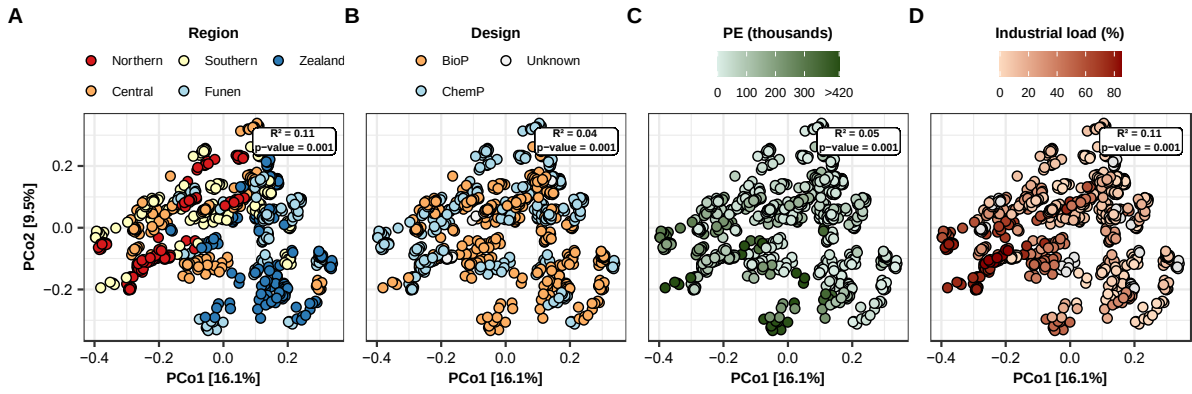


Figure S5. Effects of environmental and process parameters on the core ASV microbial community structure of activated sludge. Principal coordinate analyses of Bray–Curtis beta-diversity are calculated for core ASVs (strict, general, and loose core). Results from PERMANOVA are shown as labels. A) Regions (Northern, $n = 12$; Southern, $n = 19$; Zealand, $n = 21$; Central, $n = 19$; Funen, $n = 13$), B) Design - all plants have removal of C, N, and P. P is removed primarily with chemical precipitation (ChemP, $n = 38$) or biological removal (BioP, $n = 43$) or unknown ($n = 3$), C) persons equivalents (PE) reported in thousands (4 samples, each from Lynetten WWTP (1 mio PE), exceed 420 thousand PE). For adonis test following groups have been applied, size: Small $\leq 25,000$ PE; medium = 25,000–100,000 PE, large $> 100,000$ PE. D) percentage of industrial load in incoming wastewater (plants with unknown industrial load are colored gray). For adonis test following groups have been used, Industrial load: None = 0%, very low = 0–10%, low = 10–30%, medium = 30–50%, high 50–85%.

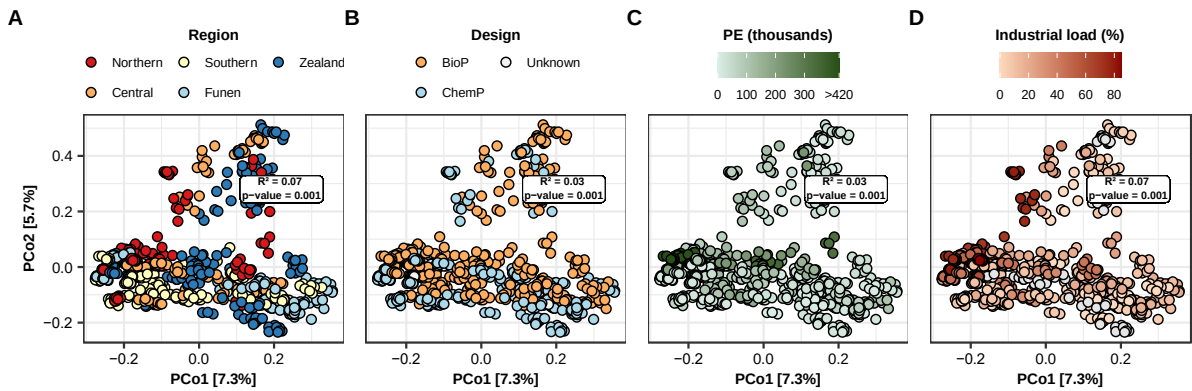


Figure S6. Effects of environmental and process parameters on the CRAT ASV microbial community structure of activated sludge. Principal coordinate analyses of Bray–Curtis beta-diversity are calculated for CRAT ASVs. Results from PERMANOVA are shown as labels. A) Regions (Northern, $n = 12$; Southern, $n = 19$; Zealand, $n = 21$; Central, $n = 19$; Funen, $n = 13$), B) Design - all plants have removal of C, N, and P. P is removed primarily with chemical precipitation (ChemP, $n = 38$) or biological removal (BioP, $n = 43$) or unknown ($n = 3$), C) persons equivalents (PE) reported in thousands (4 samples, each from Lynetten WWTP (1 mio PE), exceed 420 thousand PE). For adonis test following groups have been applied, size: Small $\leq 25,000$ PE; medium = 25,000-100,000 PE, large $> 100,000$ PE. D) percentage of industrial load in incoming wastewater (plants with unknown industrial load are colored gray). For adonis test following groups have been used, Industrial load: None = 0%, very low = 0–10%, low = 10–30%, medium = 30–50%, high 50–85%.

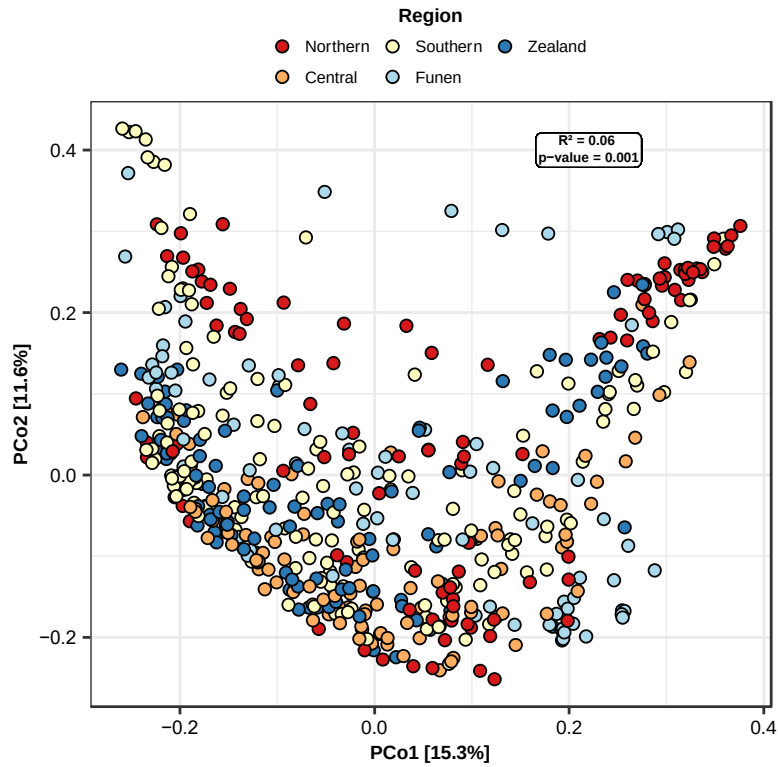


Figure S7. Community beta diversity in IWW colored by region. Beta-diversity in IWW samples of 69 plants visualized as PCoA plot based on Bray-Curtis dissimilarity matrix. The explained variance (R^2) refers to differences of communities based on region, each represented by a different color.

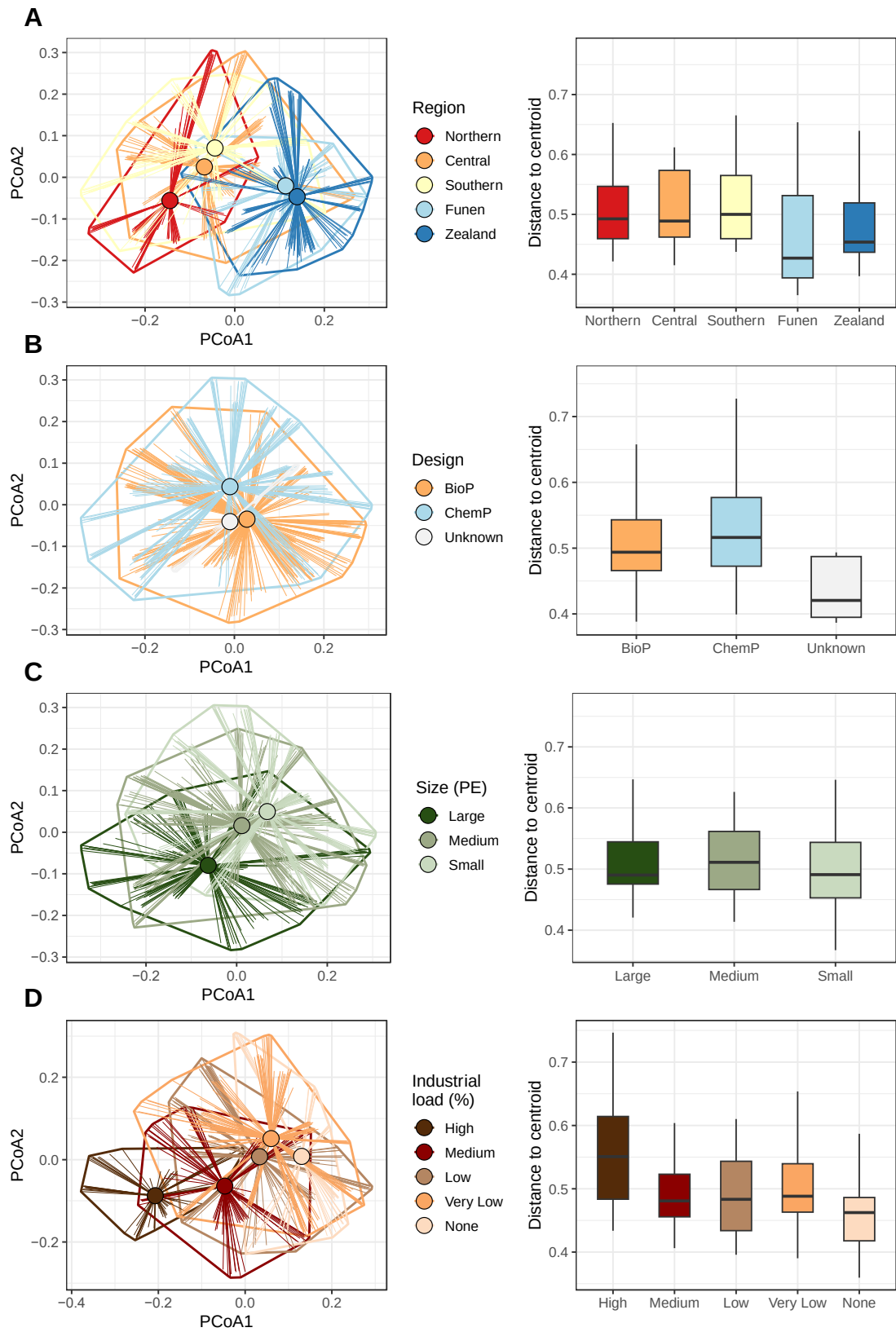


Figure S8. Multivariate homogeneity of groups dispersions (variances) done with Vegan's betadisper function in R. Left column: beta-dispersion plot with group centroids, right column: boxplot of distances to group centroid **A)** Regions (Northern, n = 12; Southern, n = 19; Zealand, n = 21; Central, n = 19; Funen, n = 13), **B)** Design - all plants have removal of C, N, and P. P is removed primarily with chemical precipitation (ChemP, n = 38) or biological removal (BioP, n = 43) or unknown (n = 3), **C)** Size evaluated by persons equivalents (PE), following groups have been applied, size: Small $\leq 25,000$ PE; medium = 25,000-100,000 PE, large $> 100,000$ PE. **D)** percentage of industrial load in incoming wastewater. Following groups have been used, industrial load: None = 0%, very low = 0–10%, low = 10–30%, medium = 30–50%, high 50–85%.

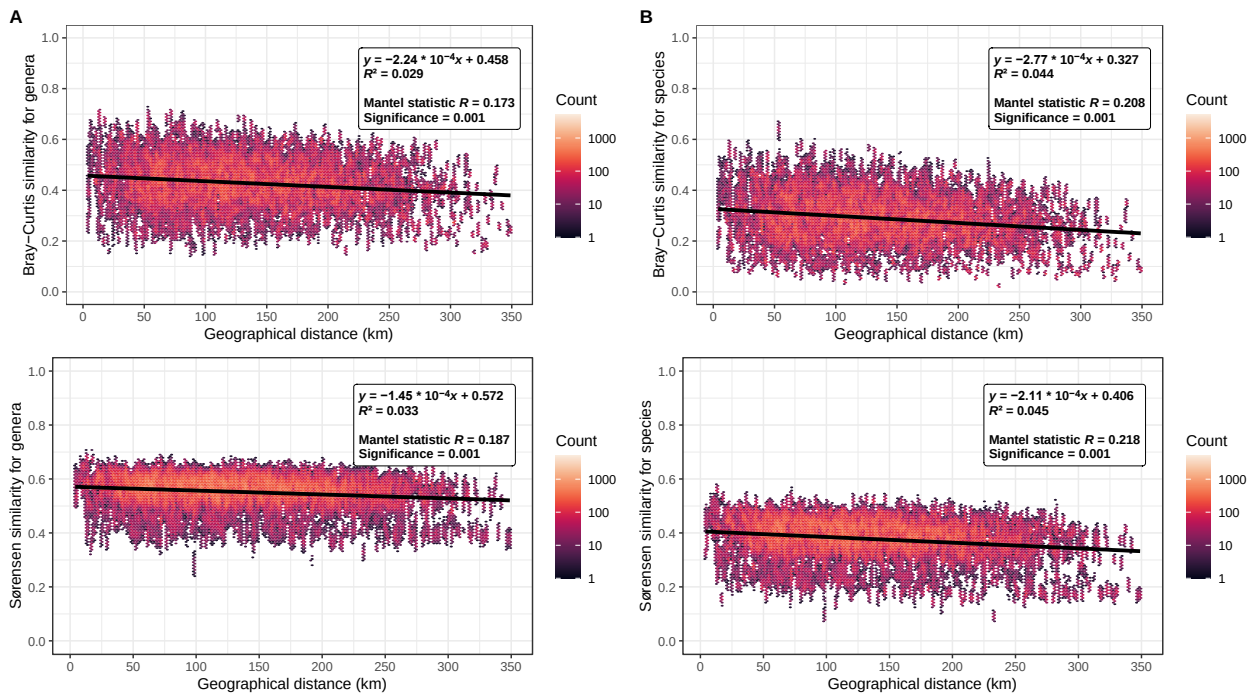


Figure S9. Distance-Decay relationship (DDR) of AS samples in all plants (n=84) based on genus and species distances. A) DDR calculated based on Bray-Curtis (weighted) dissimilarity and Sørensen (unweighted) dissimilarity at genus level. B) DDR calculated based on Bray-Curtis (weighted) dissimilarity and Sørensen (unweighted) dissimilarity at species level. Each panel shows: the statistics and significance of the Mantel test calculated using Spearman correlation and 999 permutations; the equation for the linear regression and the R^2 of the regression model. The color indicates the number of permutated distances between two samples in each bin (150 bins in both vertical and horizontal directions).

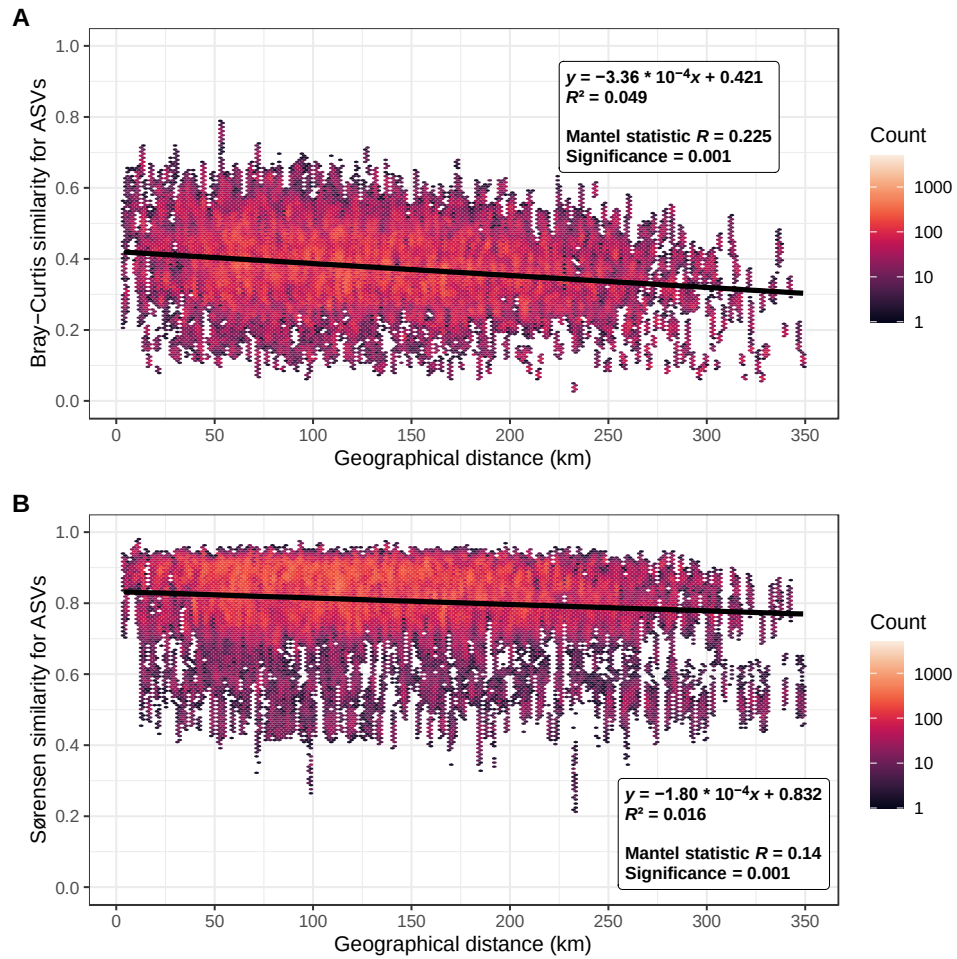


Figure S10. Distance-Decay relationship (DDR) of core ASVs in AS samples in all plants (n=84) based on ASV distances. DDR calculated based on A) Bray-Curtis (weighted) dissimilarity and B) Sørensen (unweighted) dissimilarity. Each panel shows: the statistics and significance of the Mantel test calculated using Spearman correlation and 999 permutations; the equation for the linear regression and the R^2 of the regression model. The color indicates the number of permutated distances between two samples in each bin (150 bins in both vertical and horizontal directions).

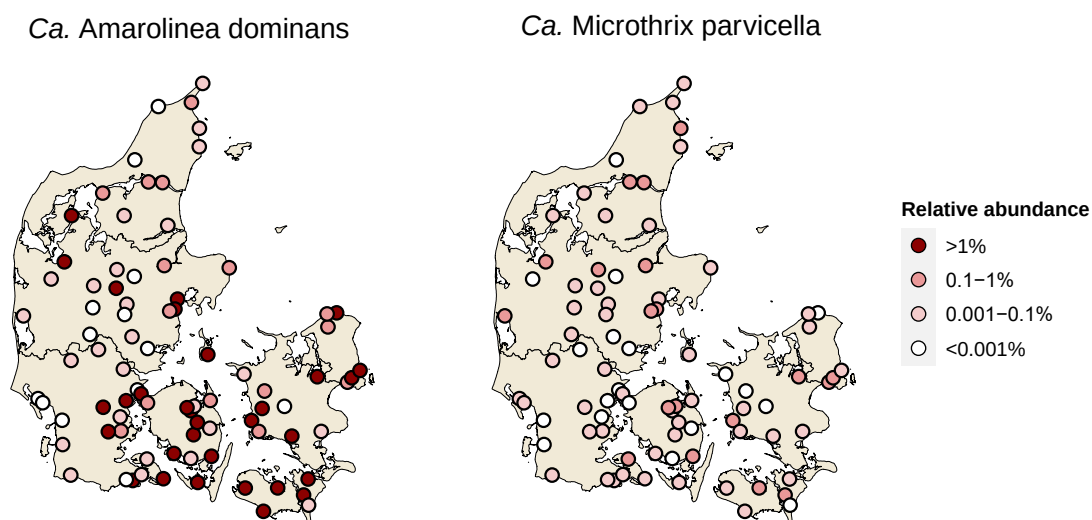


Figure S11. Geographical distribution of selected species in AS. The color of points refers to the mean relative read abundance of the filamentous *Ca. Amarolinea dominans* and *Ca. Microthrix parvicella* across samples in each WWTP.

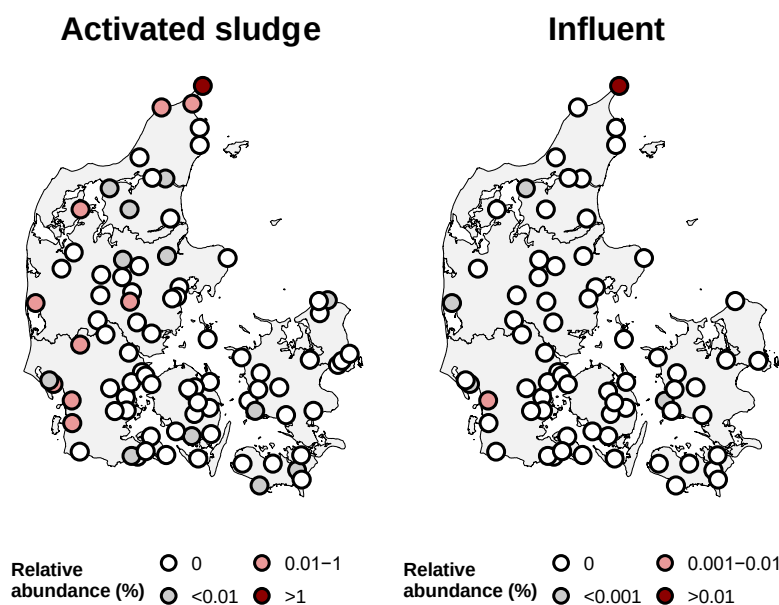


Figure S12. Geographical distribution of *Ca. Epiflobacter midas_s_452* in activated sludge (left) and influent wastewater (right). The color of points refers to the mean relative read abundance of the epiphytic *Ca. Epiflobacter midas_s_452* across samples in each IWW and WWTP.

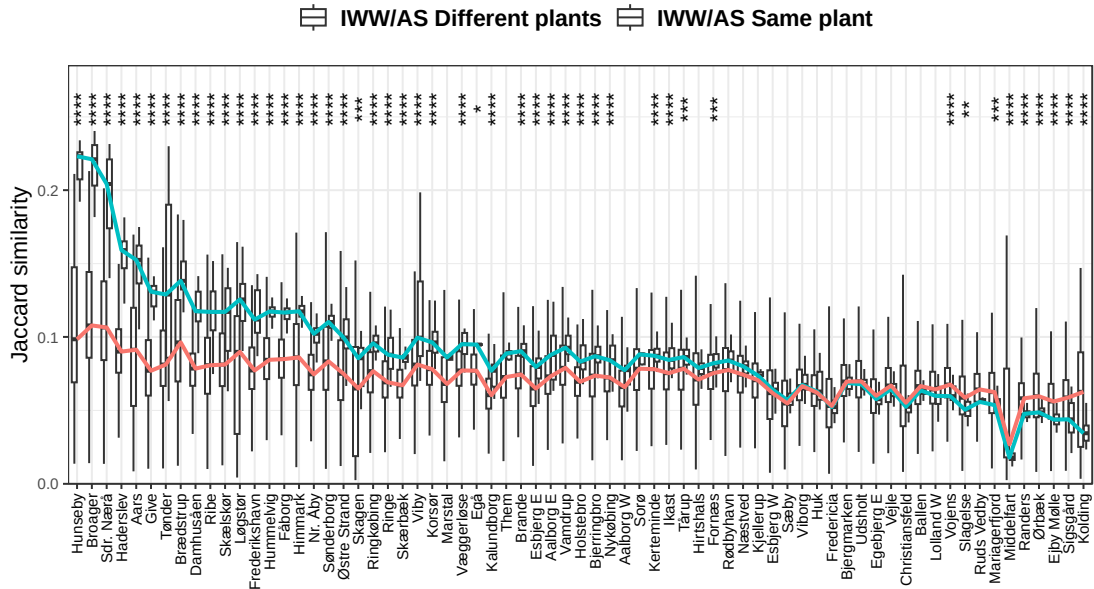


Figure S13. Similarity between IWW and AS samples from the same plant and from different plants. Boxplot of similarity between IWW and AS samples was calculated based on Jaccard matrix. On x-axis: WWTPs; on y-axis: value of similarity (1 - Jaccard dissimilarity). Colors refer to pairs of IWW and AS from the same plant and from different plants, in blue and red respectively. The WWTPs are sorted by the highest difference between median of similarity of IWW/AS pairs from the plant and from different plants. Significance of difference between means is calculated by Wilcoxon test; the significance of p-values is provided using Bonferroni correction for multiple comparisons and shown as stars (*: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$; no*: $p \geq 0.05$).