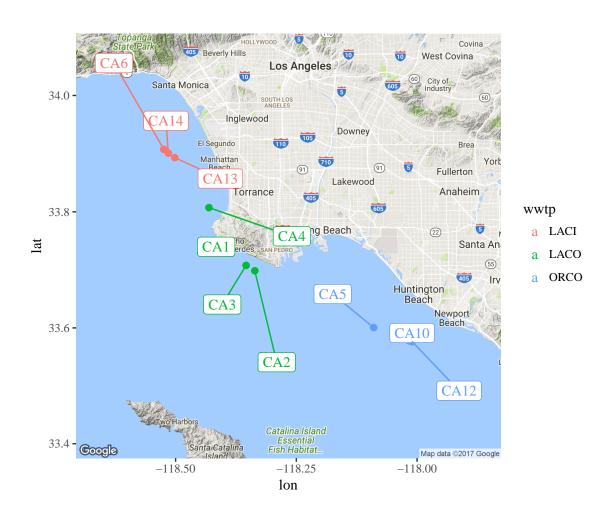
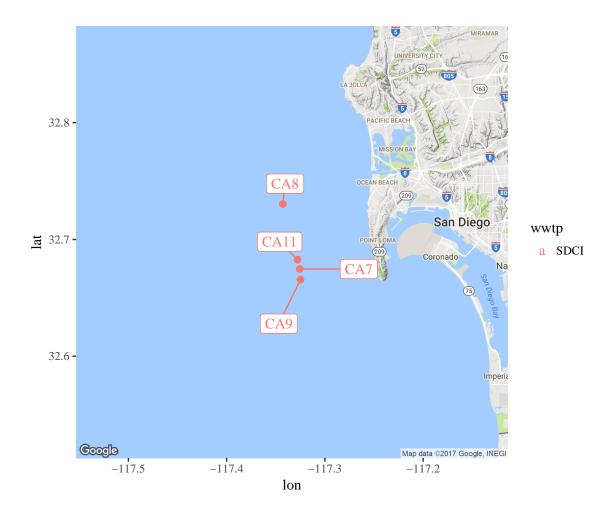
Analysis of 16S microbial sequencing data from a Southern Califoria WWTP discharge fields

August 10, 2017





```
load(file = 'data/abudat.RData')
lapply(abudat, head)
## $DOMAIN
## # A tibble: 6 x 5
## # Groups: site, wwtp, cont [6]
##
      domain site wwtp cont abund
##
       <chr> <chr> <chr> <chr> <chr> <dbl>
## 1 Archaea
                    LACO
                                  148
               CA1
                           int
## 2 Archaea CA10
                    ORCO
                           int
                                  50
## 3 Archaea CA11
                    SDCI
                           int
                                  70
## 4 Archaea CA12
                                  104
                    ORCO
                           clo
## 5 Archaea CA13
                    LACI
                           far
                                  96
## 6 Archaea CA14 LACI
                                  139
                           int
```

```
##
## $PHYLUM
## # A tibble: 6 x 6
## # Groups: site, wwtp, cont [6]
     phylum site wwtp cont abund
                                     domain
##
     <chr> <chr> <chr> <chr> <chr> <dbl>
                                      <chr>
## 1
       AC1
             CA1 LACO
                          int
                                  2 Bacteria
                                  0 Bacteria
## 2
       AC1
            CA10 ORCO
                         int
## 3
           CA11 SDCI
       AC1
                         int
                                 0 Bacteria
## 4
       AC1
           CA12 ORCO
                         clo
                                 0 Bacteria
## 5
       AC1 CA13 LACI
                         far
                                 0 Bacteria
## 6
       AC1 CA14 LACI
                         int
                                 0 Bacteria
##
## $CLASS
## # A tibble: 6 x 7
## # Groups: site, wwtp, cont [6]
             class site wwtp cont abund
##
                                            domain
                                                            phylum
##
              <chr> <chr> <chr> <chr> <chr> <dbl>
                                              <chr>
## 1 028H05-P-BN-P5
                     CA1 LACO
                                  int
                                         O Bacteria Planctomycetes
## 2 028H05-P-BN-P5
                   CA10 ORCO
                                 int
                                         O Bacteria Planctomycetes
## 3 028H05-P-BN-P5 CA11 SDCI
                                         O Bacteria Planctomycetes
                                 int
## 4 028H05-P-BN-P5 CA12 ORCO
                                 clo
                                         O Bacteria Planctomycetes
## 5 028H05-P-BN-P5 CA13 LACI
                                         O Bacteria Planctomycetes
                                 far
## 6 028H05-P-BN-P5 CA14 LACI
                                 int
                                         O Bacteria Planctomycetes
##
## $ORDER
## # A tibble: 6 x 8
              site, wwtp, cont [6]
## # Groups:
##
                order site wwtp cont abund domain
                                                               phylum
                                                 <chr>
##
                <chr> <chr> <chr> <chr> <chr> <dbl>
## 1 028H05-P-BN-P5_or
                        CA1 LACO
                                    int
                                            O Bacteria Planctomycetes
## 2 028H05-P-BN-P5_or CA10 ORCO
                                            O Bacteria Planctomycetes
                                    int
## 3 028H05-P-BN-P5_or CA11 SDCI
                                           O Bacteria Planctomycetes
                                    int
## 4 028H05-P-BN-P5_or CA12 ORCO
                                           O Bacteria Planctomycetes
                                    clo
## 5 028H05-P-BN-P5_or CA13 LACI
                                    far
                                           O Bacteria Planctomycetes
## 6 028H05-P-BN-P5_or CA14 LACI
                                           O Bacteria Planctomycetes
                                     int
## # ... with 1 more variables: class <chr>
##
## $FAMILY
## # A tibble: 6 x 9
## # Groups: site, wwtp, cont [6]
     family site wwtp cont abund
                                                     phylum
                                      domain
                                                                       class
      <chr> <chr> <chr> <chr> <chr> <dbl>
                                        <chr>
                                                      <chr>
                                                                       <chr>>
## 1 01D2Z36 CA1 LACO
                                  O Bacteria Verrucomicrobia Spartobacteria
                          int
```

```
## 2 01D2Z36 CA10
                    ORCO
                         int O Bacteria Verrucomicrobia Spartobacteria
## 3 01D2Z36 CA11
                                   O Bacteria Verrucomicrobia Spartobacteria
                    SDCI
                           int
                                   O Bacteria Verrucomicrobia Spartobacteria
## 4 01D2Z36 CA12
                    ORCO
                           clo
                                   O Bacteria Verrucomicrobia Spartobacteria
## 5 01D2Z36 CA13
                   LACI
                           far
## 6 01D2Z36 CA14
                   LACI
                           int
                                   1 Bacteria Verrucomicrobia Spartobacteria
## # ... with 1 more variables: order <chr>
##
## $GENUS
## # A tibble: 6 x 10
## # Groups: site, wwtp, cont [6]
          genus site wwtp cont abund
                                          domain
                                                          phylum
##
          <chr> <chr> <chr> <chr> <chr> <dbl>
                                           <chr>
                                                           <chr>
## 1 01D2Z36_ge
                 CA1
                      LACO
                              int
                                      O Bacteria Verrucomicrobia
## 2 01D2Z36_ge CA10
                                      O Bacteria Verrucomicrobia
                       ORCO
                              int
## 3 01D2Z36_ge CA11
                       SDCI
                                      O Bacteria Verrucomicrobia
                              int
## 4 01D2Z36_ge CA12
                       ORCO
                              clo
                                      O Bacteria Verrucomicrobia
## 5 01D2Z36_ge CA13
                                      O Bacteria Verrucomicrobia
                      LACI
                              far
## 6 01D2Z36_ge CA14 LACI
                                      1 Bacteria Verrucomicrobia
                              int
## # ... with 3 more variables: class <chr>, order <chr>, family <chr>
##
## $SPECIES
## # A tibble: 6 x 11
## # Groups:
               site, wwtp, cont [6]
      species site wwtp cont abund
                                        domain
                                                   phylum
                                                            class
       <chr> <chr> <chr> <chr> <chr> <dbl>
                                         <chr>
                                                    <chr>
                                                            <chr>
##
                                                                       <chr>>
## 1 Otu00001
                CA1 LACO
                                 4993 Bacteria Firmicutes Bacilli Bacillales
## 2 Otu00001
              CA10 ORCO
                            int 7001 Bacteria Firmicutes Bacilli Bacillales
                            int 2573 Bacteria Firmicutes Bacilli Bacillales
## 3 Otu00001
              CA11 SDCI
## 4 Otu00001
               CA12 ORCO
                            clo
                                 703 Bacteria Firmicutes Bacilli Bacillales
## 5 Otu00001
               CA13 LACI
                            far
                                  842 Bacteria Firmicutes Bacilli Bacillales
## 6 Otu00001 CA14 LACI
                            int 1578 Bacteria Firmicutes Bacilli Bacillales
## # ... with 2 more variables: family <chr>, genus <chr>
```

1 Community structure

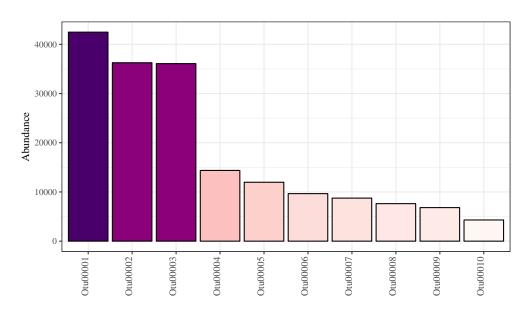


Figure 1: Top fifty most abundant species across all study sites.

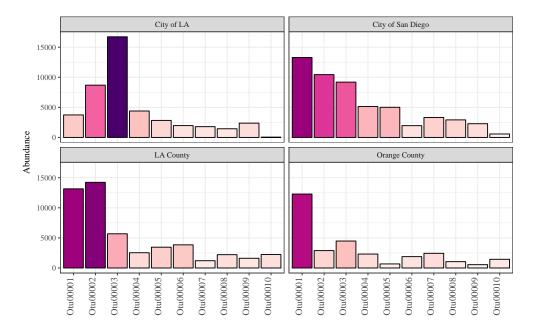


Figure 2: Species abundances by municipal locations using the top fifty most abundant across all study sites in fig. 1.

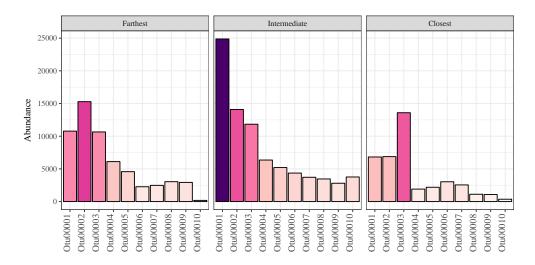


Figure 3: Species abundances by distance from pipe for the top fifty most abundant across all study sites in fig. 1. Treatments are based on relative distances from an outflow pipe (farthest, intermediate, closest) for each municipality.

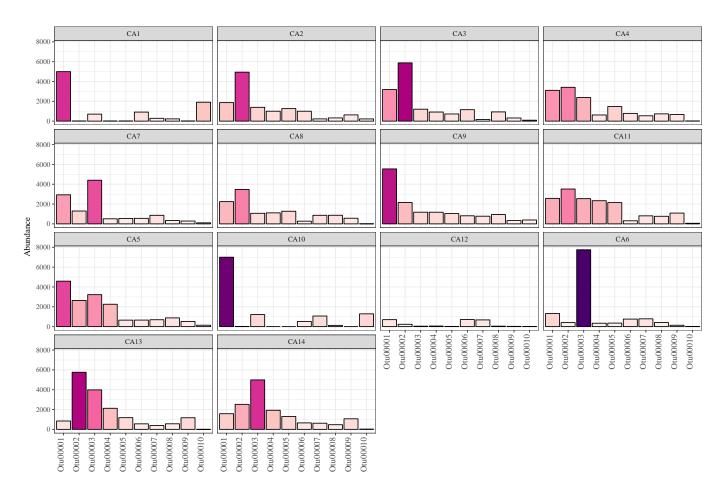


Figure 4: Species abundances by sites using the top fifty most abundant across all study sites in fig. 1.

1.1 Multivariate analyses with archaea

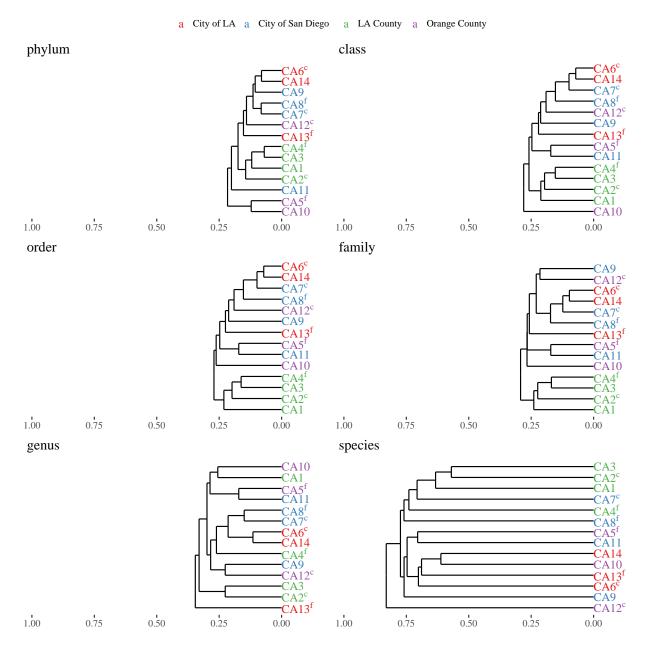


Figure 5: Site clusters of archaea by taxonomic resolution. Colors indicate municipality and superscripts indicate distance categories from an outflow pipe at each site ('f' is farthest, 'c' is closest, none is intermediate). Clustering was based on a Bray-Curtis dissimilarity matrix of abundance data and sorting using the unweighted pair group method.

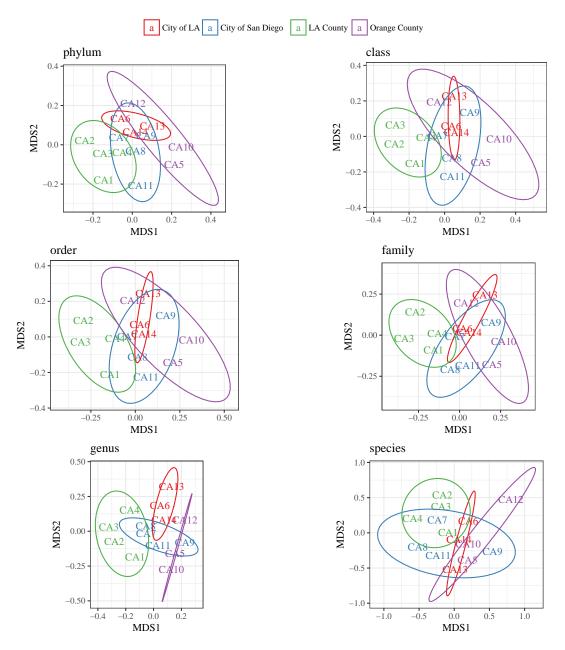


Figure 6: Site ordinations of archaea by taxonomic resolution. Colors indicate municipality with ellipses showing 95% bivariate confidence intervals. Ordinations were created using Nonmetric Multidimensional scaling with several random starts to find a stable solution of the configuration. A Bray-Curtis dissimilarity matrix was used as a measure of differences between sites. The ordination is the same as in fig. 7 but with different group categories in the plot.

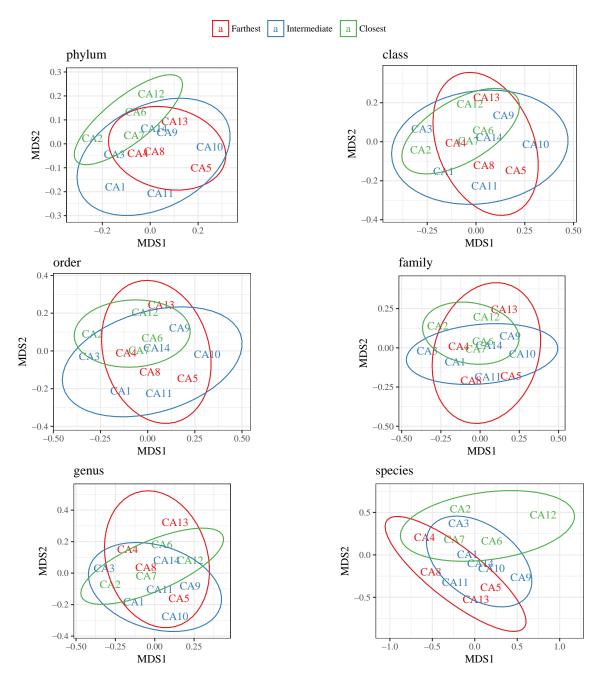


Figure 7: Site ordinations of archaea by taxonomic resolution. Colors indicate approximate distance from pipe with ellipses showing 95% bivariate confidence intervals. Ordinations were created using Nonmetric Multidimensional scaling with several random starts to find a stable solution of the configuration. A Bray-Curtis dissimilarity matrix was used as a measure of differences between sites. The ordination is the same as in fig. 6 but with different group categories in the plot.

Table 1: Alpha diversity of archaea by municipality, distance from outflow, and sites for each taxonomic level. Original data were taxanomic abundance aggregated by each grouping. Alpha was was based on methods in Fisher et al. (1943) that measure diversity as a function of richness and abundance at a site.

Location grouping	Phylum	Class	Order	Family	Genus	Species
Municipality						
City of LA	1.83	2.77	3.02	3.80	4.62	70.34
City of San Diego	1.78	2.70	2.70	3.69	3.95	135.86
LA County	2.07	3.16	3.63	4.10	4.85	80.58
Orange County	1.65	2.47	2.47	3.06	3.37	88.71
Pipe location						
Farthest	2.06	3.02	3.28	4.34	5.19	109.98
Intermediate	2.11	3.00	3.47	3.95	4.70	88.42
Closest	1.63	2.44	2.65	3.31	4.00	127.81
Site						
CA1	2.11	2.75	2.75	2.75	2.75	23.76
CA2	2.00	2.79	3.07	3.35	3.64	46.23
CA3	2.19	3.58	3.96	3.96	3.96	27.14
CA4	1.67	2.33	2.33	2.68	3.44	44.31
CA7	1.49	2.35	2.35	2.66	2.98	86.56
CA8	1.22	1.75	1.75	2.33	2.33	53.49
CA9	1.50	2.59	2.59	3.00	3.44	29.57
CA11	3.19	3.67	3.67	4.17	4.17	47.43
CA5	2.10	3.01	3.01	3.51	3.51	45.68
CA10	1.78	2.69	2.69	2.69	2.69	23.95
CA12	1.69	2.73	2.73	3.51	3.92	59.12
CA6	1.42	2.23	2.23	2.23	3.14	34.14
CA13	2.43	4.06	4.51	6.00	7.10	38.20
CA14	1.28	2.15	2.15	2.47	3.15	30.15

Table 2: Beta diversity of archaea between municipalities, distance categories from outflow, and sites for each taxonomic level. Original data were taxonomic abundance aggregated by each grouping. Beta was estimated as total species richness across all categories in each grouping, divided by mean species richness within each category, minus one.

Location grouping	Phylum	Class	Order	Family	Genus	Species
Distance from outflow	0.18	0.27	0.35	0.45	0.48	1.39
Site	0.72	0.86	1.11	1.45	1.67	7.64
Municipality	0.30	0.36	0.49	0.60	0.69	2.05

1.2 Multivariate analyses with bacteria

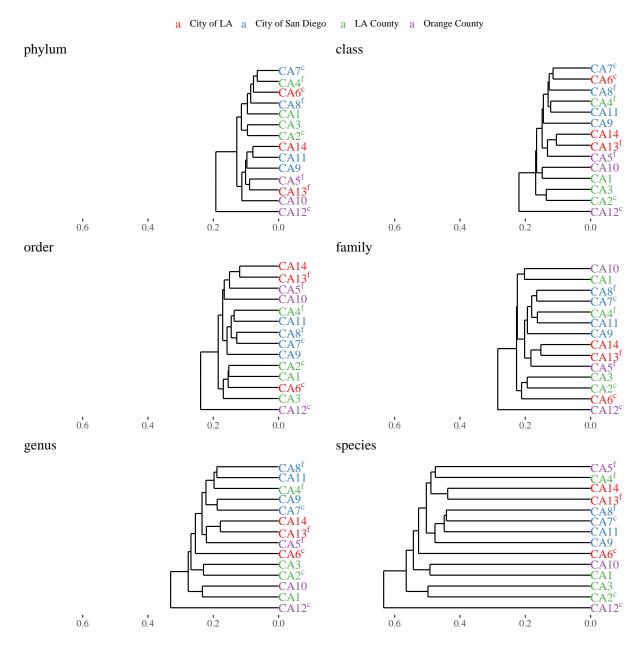


Figure 8: Site clusters of bacteria by taxonomic resolution. Colors indicate municipality and superscripts indicate distance categories from an outflow pipe at each site ('f' is farthest, 'c' is closest, none is intermediate). Clustering was based on a Bray-Curtis dissimilarity matrix of abundance data and sorting using the unweighted pair group method.

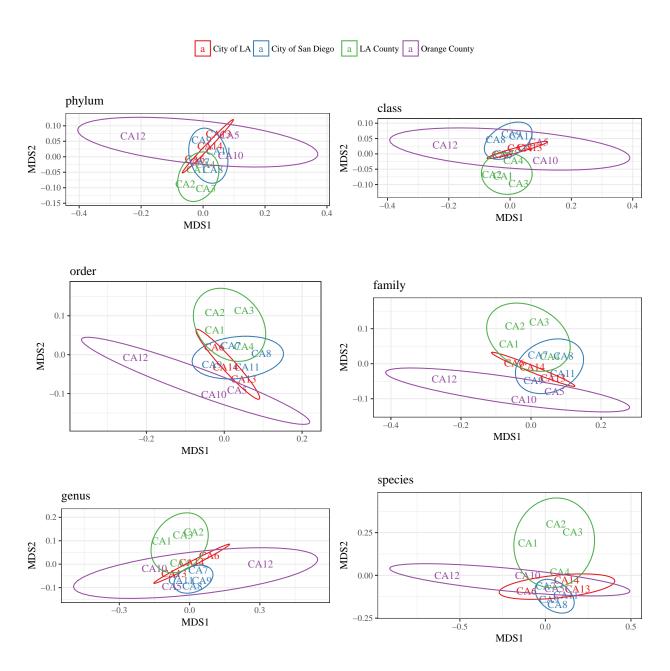


Figure 9: Site ordinations of bacteria by taxonomic resolution. Colors indicate municipality with ellipses showing 95% bivariate confidence intervals. Ordinations were created using Nonmetric Multidimensional scaling with several random starts to find a stable solution of the configuration. A Bray-Curtis dissimilarity matrix was used as a measure of differences between sites. The ordination is the same as in fig. 10 but with different group categories in the plot.

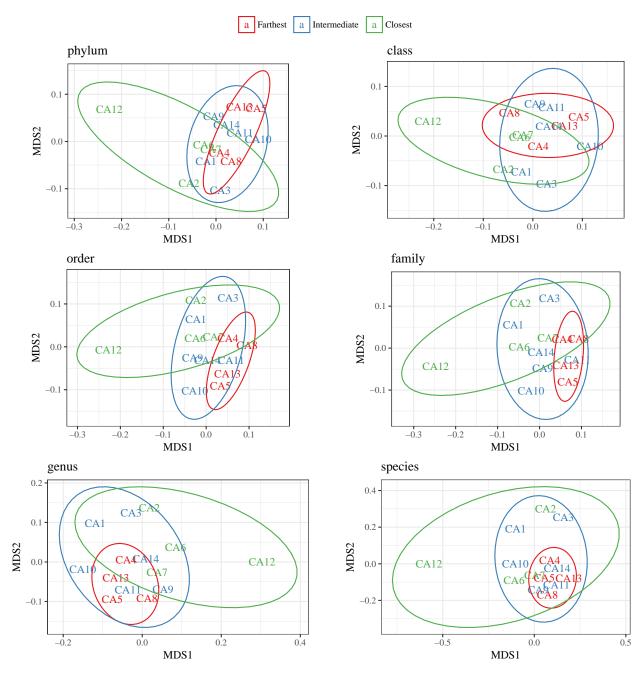


Figure 10: Site ordinations of bacteria by taxonomic resolution. Colors indicate approximate distance from pipe with ellipses showing 95% bivariate confidence intervals. Ordinations were created using Nonmetric Multidimensional scaling with several random starts to find a stable solution of the configuration. A Bray-Curtis dissimilarity matrix was used as a measure of differences between sites. The ordination is the same as in fig. 9 but with different group categories in the plot.

Table 3: Alpha diversity of bacteria by municipality, distance from outflow, and sites for each taxonomic level. Original data were taxanomic abundance aggregated by each grouping. Alpha was was based on methods in Fisher et al. (1943) that measure diversity as a function of richness and abundance at a site.

Location grouping	Phylum	Class	Order	Family	Genus	Species
Municipality						
City of LA	5.28	16.57	30.81	54.53	93.88	1161.57
City of San Diego	5.45	16.99	31.80	57.76	98.51	1334.51
LA County	5.68	17.89	33.21	58.85	104.05	1626.50
Orange County	4.94	16.30	28.77	51.04	89.02	1039.74
Pipe location						
Farthest	5.11	16.34	30.26	52.45	83.41	1261.77
Intermediate	5.53	17.49	32.48	59.57	108.56	1680.25
Closest	5.45	17.90	34.49	62.40	117.12	1756.89
Site						
CA1	5.26	17.23	29.40	50.32	78.76	785.92
CA2	6.06	18.51	34.29	57.25	95.48	841.61
CA3	5.66	15.83	29.23	46.20	74.05	637.01
CA4	5.39	15.98	27.35	43.05	63.42	692.85
CA7	5.52	16.61	30.61	50.89	81.67	731.89
CA8	5.79	17.23	29.92	48.82	73.05	791.90
CA9	5.26	15.20	27.85	48.60	80.37	571.54
CA11	4.99	14.90	26.00	42.68	64.79	544.88
CA5	4.09	13.07	24.00	39.41	57.16	448.07
CA10	4.21	13.07	24.00	39.58	59.67	501.72
CA12	5.13	15.98	27.18	46.19	78.12	639.99
CA6	5.79	17.08	31.48	53.75	88.60	857.85
CA13	4.47	14.29	25.83	41.41	62.04	464.25
CA14	4.86	15.21	26.85	44.90	72.63	570.57

Table 4: Beta diversity of bacteria between municipalities, distance categories from outflow, and sites for each taxonomic level. Original data were taxonomic abundance aggregated by each grouping. Beta was estimated as total species richness across all categories in each grouping, divided by mean species richness within each category, minus one.

Location grouping	Phylum	Class	Order	Family	Genus	Species
Distance from outflow	0.07	0.13	0.18	0.23	0.37	1.02
Site	0.31	0.48	0.63	0.85	1.31	4.72
Municipality	0.11	0.18	0.26	0.33	0.51	1.50