

# More diverse school microbiota may provide better protection against respiratory infections for school staff

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Table S1. Summary of the operational taxonomic units (OTUs) in the original 499 samples, categorized by bacterial and fungal groups

| Bacterial and Fungal Groups          | Statistics, N = 499 |
|--------------------------------------|---------------------|
| Total bacteria                       |                     |
| Median (75 <sup>th</sup> percentile) | 415 (492)           |
| Range                                | 70–700              |
| Gram positive bacteria               |                     |
| Median (75 <sup>th</sup> percentile) | 210 (244)           |
| Range                                | 37–367              |
| Actinobacteria                       |                     |
| Median (75 <sup>th</sup> percentile) | 113 (130)           |
| Range                                | 14–186              |
| Firmicutes                           |                     |
| Median (75 <sup>th</sup> percentile) | 100 (117)           |
| Range                                | 12–193              |
| Gram negative bacteria               |                     |
| Median (75 <sup>th</sup> percentile) | 208 (246)           |
| Range                                | 33–383              |
| Total fungi                          |                     |
| Median (75 <sup>th</sup> percentile) | 163 (202)           |
| Range                                | 13–325              |
| Yeasts                               |                     |
| Median (75 <sup>th</sup> percentile) | 26 (32)             |
| Range                                | 2–52                |

Table S2. Unadjusted prevalence of infection reported in the past 12 months by the exposure group based on total bacteria richness (the fourth quartile, Q4 versus reference group, the lower three quartiles, Q123 in the number of OTUs)

| Type of Infection      | Total Bacteria           |                              | Difference <sup>2</sup> | 95% CI <sup>2,3</sup> | P-value <sup>2</sup> |
|------------------------|--------------------------|------------------------------|-------------------------|-----------------------|----------------------|
|                        | Q4, N = 353 <sup>1</sup> | Q123, N = 1,060 <sup>1</sup> |                         |                       |                      |
| Acute bronchitis       | 50/329 (15%)             | 187/945 (20%)                | -4.6%                   | -9.4%, 0.25%          | 0.078                |
| Pneumonia              | 13/333 (3.9%)            | 44/952 (4.6%)                | -0.72%                  | -3.4%, 2.0%           | 0.7                  |
| Influenza-like illness | 162/333 (49%)            | 521/958 (54%)                | -5.7%                   | -12%, 0.69%           | 0.081                |
| Common cold            | 201/331 (61%)            | 628/950 (66%)                | -5.4%                   | -12%, 0.88%           | 0.090                |
| Nose infection         | 162/333 (49%)            | 514/955 (54%)                | -5.2%                   | -12%, 1.3%            | 0.12                 |
| Sinus infection        | 168/329 (51%)            | 542/955 (57%)                | -5.7%                   | -12%, 0.76%           | 0.084                |
| Throat infection       | 159/329 (48%)            | 482/951 (51%)                | -2.4%                   | -8.8%, 4.1%           | 0.5                  |
| Ear infection          | 81/329 (25%)             | 235/952 (25%)                | -0.06%                  | -5.5%, 5.4%           | >0.9                 |
| Vomiting               | 48/332 (14%)             | 170/952 (18%)                | -3.4%                   | -8.1%, 1.3%           | 0.2                  |
| Diarrhea               | 103/334 (31%)            | 356/953 (37%)                | -6.5%                   | -13%, -0.49%          | 0.038                |
| Nausea                 | 95/335 (28%)             | 326/951 (34%)                | -5.9%                   | -12%, -0.03%          | 0.055                |

<sup>1</sup> n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure were excluded in the analyses. Thus, the total number of observations for this analysis is 1,413; <sup>2</sup>Two sample test for equality of proportions; <sup>3</sup>CI = Confidence Interval.

Table S3. Prevalence of infection reported in the past 12 months by the exposure to total fungi

| Type of Infection      | Total Fungi              |                              | Difference <sup>2</sup> | 95% CI <sup>2,3</sup> | P-value <sup>2</sup> |
|------------------------|--------------------------|------------------------------|-------------------------|-----------------------|----------------------|
|                        | Q4, N = 353 <sup>1</sup> | Q123, N = 1,060 <sup>1</sup> |                         |                       |                      |
| Acute bronchitis       | 57/322 (18%)             | 180/952 (19%)                | -1.2%                   | -6.3%, 3.9%           | 0.7                  |
| Pneumonia              | 11/323 (3.4%)            | 46/962 (4.8%)                | -1.4%                   | -4.0%, 1.2%           | 0.4                  |
| Influenza-like illness | 165/322 (51%)            | 518/969 (53%)                | -2.2%                   | -8.7%, 4.3%           | 0.5                  |
| Common cold            | 203/325 (62%)            | 626/956 (65%)                | -3.0%                   | -9.3%, 3.3%           | 0.4                  |
| Nose infection         | 158/327 (48%)            | 518/961 (54%)                | -5.6%                   | -12%, 0.89%           | 0.092                |
| Sinus infection        | 168/325 (52%)            | 542/959 (57%)                | -4.8%                   | -11%, 1.7%            | 0.15                 |
| Throat infection       | 151/325 (46%)            | 490/955 (51%)                | -4.8%                   | -11%, 1.6%            | 0.15                 |
| Ear infection          | 65/323 (20%)             | 251/958 (26%)                | -6.1%                   | -11%, -0.69%          | 0.034                |
| Vomiting               | 66/321 (21%)             | 152/963 (16%)                | 4.8%                    | -0.42%, 10%           | 0.059                |
| Diarrhea               | 118/325 (36%)            | 341/962 (35%)                | 0.86%                   | -5.4%, 7.1%           | 0.8                  |
| Nausea                 | 112/323 (35%)            | 309/963 (32%)                | 2.6%                    | -3.6%, 8.8%           | 0.4                  |

<sup>1</sup> n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure were excluded in the analyses. Thus, the total number of observations for this analysis is 1,413; <sup>2</sup>Two sample test for equality of proportions; <sup>3</sup>CI = Confidence Interval.

Table S4. Prevalence of infection reported in the past 12 months by the exposure to yeasts

| Type of Infection      | Yeasts                   |                              | Difference <sup>2</sup> | 95% CI <sup>2,3</sup> | P-value <sup>2</sup> |
|------------------------|--------------------------|------------------------------|-------------------------|-----------------------|----------------------|
|                        | Q4, N = 353 <sup>1</sup> | Q123, N = 1,060 <sup>1</sup> |                         |                       |                      |
| Acute bronchitis       | 50/308 (16%)             | 187/966 (19%)                | -3.1%                   | -8.2%, 1.9%           | 0.3                  |
| Pneumonia              | 15/311 (4.8%)            | 42/974 (4.3%)                | 0.51%                   | -2.4%, 3.4%           | 0.8                  |
| Influenza-like illness | 165/311 (53%)            | 518/980 (53%)                | 0.20%                   | -6.4%, 6.8%           | >0.9                 |
| Common cold            | 196/310 (63%)            | 633/971 (65%)                | -2.0%                   | -8.3%, 4.4%           | 0.6                  |
| Nose infection         | 157/314 (50%)            | 519/974 (53%)                | -3.3%                   | -9.9%, 3.3%           | 0.3                  |
| Sinus infection        | 165/312 (53%)            | 545/972 (56%)                | -3.2%                   | -9.8%, 3.4%           | 0.4                  |
| Throat infection       | 151/311 (49%)            | 490/969 (51%)                | -2.0%                   | -8.6%, 4.6%           | 0.6                  |
| Ear infection          | 72/310 (23%)             | 244/971 (25%)                | -1.9%                   | -7.6%, 3.7%           | 0.5                  |
| Vomiting               | 60/309 (19%)             | 158/975 (16%)                | 3.2%                    | -2.0%, 8.4%           | 0.2                  |
| Diarrhea               | 106/313 (34%)            | 353/974 (36%)                | -2.4%                   | -8.6%, 3.9%           | 0.5                  |
| Nausea                 | 110/313 (35%)            | 311/973 (32%)                | 3.2%                    | -3.1%, 9.4%           | 0.3                  |

<sup>1</sup> n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure were excluded in the analyses. Thus, the total number of observations for this analysis is 1,413; <sup>2</sup>Two sample test for equality of proportions; <sup>3</sup>CI = Confidence Interval.

Table S5. Adjusted odds ratio (OR) and 95% confidence interval (CI) of bacterial richness in floor dust (4<sup>th</sup> quartile versus lower three quartiles in the number of OTUs) for gastrointestinal infections in the past 12 months

| Type of Infections | Type of bacteria           | Adjusted OR (95% CI) | P-value |
|--------------------|----------------------------|----------------------|---------|
| Vomiting           |                            |                      |         |
|                    | Total bacteria             | 0.81 (0.54–1.20)     | 0.31    |
|                    | Gram-positive <sup>1</sup> | 0.80 (0.53–1.19)     | 0.28    |
|                    | Actinobacteria             | 0.97 (0.65–0.43)     | 0.89    |
|                    | Firmicutes                 | 0.85 (0.57–1.25)     | 0.41    |
|                    | Gram-negative              | 0.89 (0.60–1.31)     | 0.56    |
| Diarrhea           |                            |                      |         |
|                    | Total bacteria             | 0.76 (0.55–1.03)     | 0.08    |
|                    | Gram-positive              | 0.77 (0.56–1.06)     | 0.11    |
|                    | Actinobacteria             | 1.01 (0.74–1.37)     | 0.97    |
|                    | Firmicutes                 | 0.84 (0.61–1.14)     | 0.26    |
|                    | Gram-negative              | 0.81 (0.59–1.10)     | 0.17    |
| Nausea             |                            |                      |         |
|                    | Total bacteria             | 0.78 (0.57–1.08)     | 0.14    |
|                    | Gram-positive              | 0.75 (0.54–1.04)     | 0.09    |
|                    | Actinobacteria             | 0.82 (0.59–1.12)     | 0.22    |
|                    | Firmicutes                 | 0.84 (0.61–1.16)     | 0.30    |
|                    | Gram-negative              | 0.87 (0.63–1.20)     | 0.40    |

The logistic regression models were adjusted for age, sex, race, ethnicity, current smoker, being a teacher, current asthma, hay fever, and home mold odor.

<sup>1</sup> Gram-positive bacteria included two phyla- Actinobacteria and Firmicutes.

Table S6. Prevalence of infection reported in the past 12 months by having physician-diagnosed hay fever

| Type of Infection      | Physician-diagnosed Hay Fever |                          | Difference <sup>2</sup> | 95% CI <sup>2,3</sup> | P-value <sup>2</sup> |
|------------------------|-------------------------------|--------------------------|-------------------------|-----------------------|----------------------|
|                        | Yes, N = 767 <sup>1</sup>     | No, N = 593 <sup>1</sup> |                         |                       |                      |
| Acute bronchitis       | 168/749 (22%)                 | 79/584 (14%)             | 8.9%                    | 4.7%, 13%             | <0.001               |
| Pneumonia              | 35/753 (4.6%)                 | 23/590 (3.9%)            | 0.75%                   | -1.6%, 3.1%           | 0.6                  |
| Influenza-like illness | 451/761 (59%)                 | 261/590 (44%)            | 15%                     | 9.6%, 20%             | <0.001               |
| Common cold            | 541/756 (72%)                 | 324/590 (55%)            | 17%                     | 11%, 22%              | <0.001               |
| Nose infection         | 471/762 (62%)                 | 235/589 (40%)            | 22%                     | 17%, 27%              | <0.001               |
| Sinus infection        | 521/760 (69%)                 | 220/589 (37%)            | 31%                     | 26%, 36%              | <0.001               |
| Throat infection       | 438/758 (58%)                 | 227/585 (39%)            | 19%                     | 14%, 24%              | <0.001               |
| Ear infection          | 236/759 (31%)                 | 96/586 (16%)             | 15%                     | 10%, 19%              | <0.001               |
| Vomiting               | 162/757 (21%)                 | 67/585 (11%)             | 10%                     | 5.9%, 14%             | <0.001               |
| Diarrhea               | 319/760 (42%)                 | 158/587 (27%)            | 15%                     | 9.9%, 20%             | <0.001               |
| Nausea                 | 299/756 (40%)                 | 138/589 (23%)            | 16%                     | 11%, 21%              | <0.001               |

<sup>1</sup> n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure and additional 53 who did not answer the question about physician-diagnosed hay fever were excluded in the analyses. Thus, the total number of observations for this analysis is 1,360; <sup>2</sup>Two sample test for equality of proportions; <sup>3</sup>CI = Confidence Interval.

Table S7. Prevalence of infection reported in the past 12 months by having current asthma

| Type of Infection      | Current asthma            |                            | Difference <sup>2</sup> | 95% CI <sup>2,3</sup> | P-value <sup>2</sup> |
|------------------------|---------------------------|----------------------------|-------------------------|-----------------------|----------------------|
|                        | Yes, N = 211 <sup>1</sup> | No, N = 1,134 <sup>1</sup> |                         |                       |                      |
| Acute bronchitis       | 73/206 (35%)              | 169/1,112 (15%)            | 20%                     | 13%, 27%              | <0.001               |
| Pneumonia              | 21/208 (10%)              | 36/1,119 (3.2%)            | 6.9%                    | 2.4%, 11%             | <0.001               |
| Influenza-like illness | 139/211 (66%)             | 564/1,124 (50%)            | 16%                     | 8.4%, 23%             | <0.001               |
| Common cold            | 161/209 (77%)             | 689/1,121 (61%)            | 16%                     | 8.9%, 22%             | <0.001               |
| Nose infection         | 151/211 (72%)             | 547/1,125 (49%)            | 23%                     | 16%, 30%              | <0.001               |
| Sinus infection        | 153/209 (73%)             | 579/1,124 (52%)            | 22%                     | 15%, 29%              | <0.001               |
| Throat infection       | 130/208 (62%)             | 526/1,120 (47%)            | 16%                     | 8.1%, 23%             | <0.001               |
| Ear infection          | 83/207 (40%)              | 241/1,123 (21%)            | 19%                     | 11%, 26%              | <0.001               |
| Vomiting               | 57/208 (27%)              | 169/1,118 (15%)            | 12%                     | 5.6%, 19%             | <0.001               |
| Diarrhea               | 91/208 (44%)              | 378/1,123 (34%)            | 10%                     | 2.5%, 18%             | 0.007                |
| Nausea                 | 93/209 (44%)              | 338/1,120 (30%)            | 14%                     | 6.8%, 22%             | <0.001               |

<sup>1</sup> n/N (%) for the rest of the binary variables; 116 people who did not assigned exposure and additional 68 who did not answer the question about having current asthma were excluded in the analyses. Thus, the total number of observations for this analysis is 1,345; <sup>2</sup>Two sample test for equality of proportions; <sup>3</sup>CI = Confidence Interval.

Table S8. Odds ratios (95% CI) of all variables in the full model for acute bronchitis and exposure to Actinobacteria richness

| Variable                                   | OR <sup>1</sup> | 95% CI <sup>1</sup> | P-value |
|--------------------------------------------|-----------------|---------------------|---------|
| Age                                        | 1.01            | 1.00, 1.03          | 0.057   |
| Sex                                        |                 |                     | 0.021   |
| Female                                     | –               | –                   |         |
| Male                                       | 0.53            | 0.28, 0.91          |         |
| Race                                       |                 |                     | 0.7     |
| Non-white                                  | –               | –                   |         |
| White                                      | 1.10            | 0.74, 1.64          |         |
| Current smoker                             |                 |                     | 0.3     |
| No                                         | –               | –                   |         |
| Yes                                        | 1.45            | 0.73, 2.72          |         |
| Teacher                                    |                 |                     | 0.023   |
| No                                         | –               | –                   |         |
| Yes                                        | 1.81            | 1.08, 3.16          |         |
| Ethnicity                                  |                 |                     | >0.9    |
| Hispanic                                   | –               | –                   |         |
| Non-Hispanic                               | 1.00            | 0.45, 2.46          |         |
| Current asthma                             |                 |                     | <0.001  |
| No                                         | –               | –                   |         |
| Yes                                        | 3.01            | 2.02, 4.49          |         |
| Physician-diagnosed hay fever              |                 |                     | 0.079   |
| No                                         | –               | –                   |         |
| Yes                                        | 1.38            | 0.96, 1.99          |         |
| Mold odor at home in the last 12 months    |                 |                     | >0.9    |
| No                                         | –               | –                   |         |
| Yes                                        | 1.01            | 0.59, 1.68          |         |
| School floor average dampness score        | 1.05            | 0.82, 1.32          | 0.7     |
| Binary in number of OTUs of Actinobacteria |                 |                     | 0.005   |
| Lower three quartiles combined (Q123)      | –               | –                   |         |
| Highest quartile (Q4)                      | 0.56            | 0.36, 0.84          |         |

<sup>1</sup>OR = Odds Ratio, CI = Confidence Interval

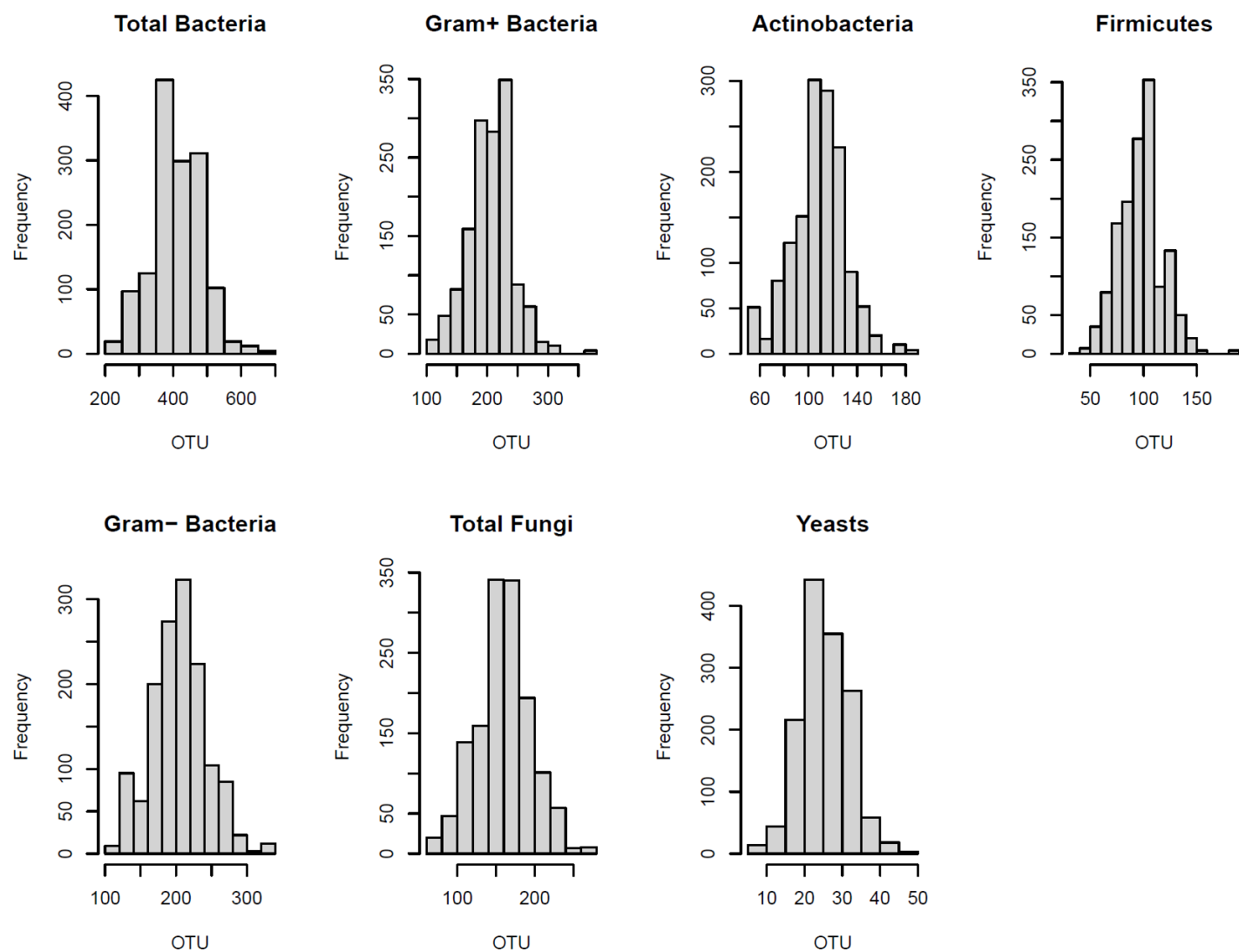


Figure S1. Distribution of floor average bacterial and fungal operational taxonomic units (OTUs) assigned to the participants (N=1,413 that excluded 116 missing in the assigned floor average values due to incomplete information on school and floor) in the health questionnaire survey.

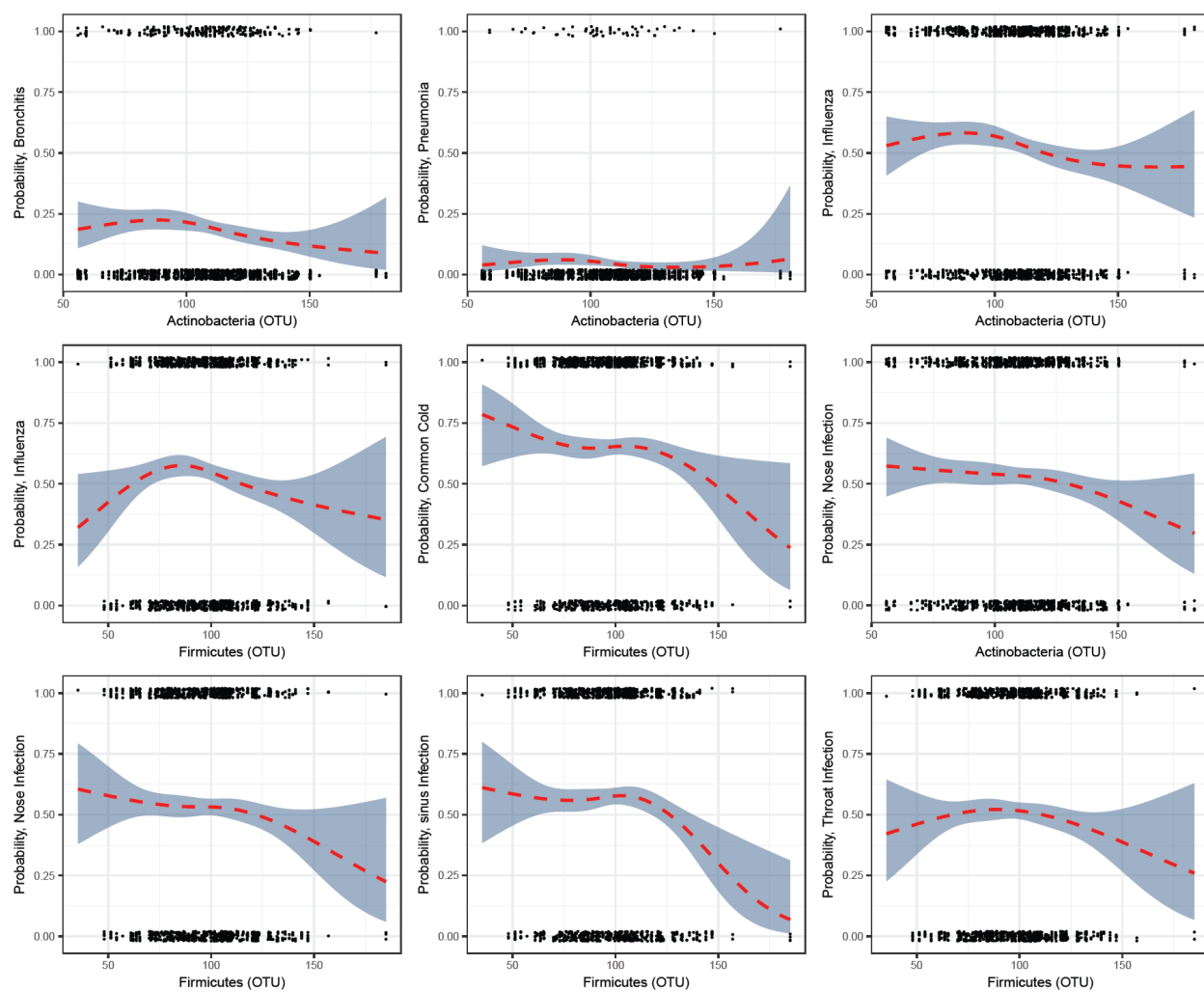


Figure S2. Unadjusted spline curve of probability of selected respiratory infections significantly associated with Actinobacteria or Firmicutes richness in a multivariable logistic regression model (Table 6).. Note that adjusted generalized additive models did not indicate non-linear exposure-response relationships, except for the sinus infection [P-value=0.025 for the non-linear function in the generalized additive models (GAM)].