

Additional file 1: Supplemental Figures and Tables for the manuscript: “The bacterial community of childcare centers: Potential implications for microbial dispersal and child exposure” by Beasley, D.E.^{1,2}, Monsur, M., Hu, J., Dunn, R.R., and Madden, A.A.

Figure S1. Occupancy of classrooms sampled in this study. Occupant density was calculated as the number of children in the class per square foot of classroom space. Each point represents a unique classroom (n=2 per center). Occupant density did not significantly differ across childcare centers.

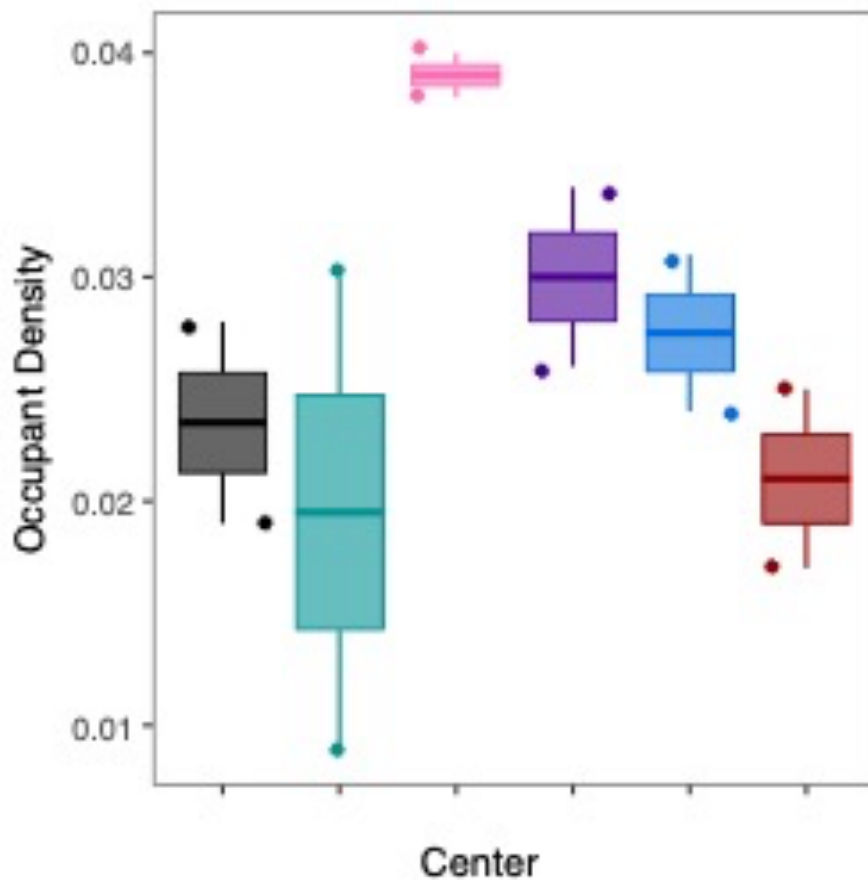


Figure S2. Measures of illumination (lux) at sampling locations across classrooms. “O” = Outside Window, “W” Inside Window, “WD” Inside Desk near Window, “DD” Inside Desk near Door, “D” Inside Door. Lowercase letters indicate statistically significant differences in illumination among samples.

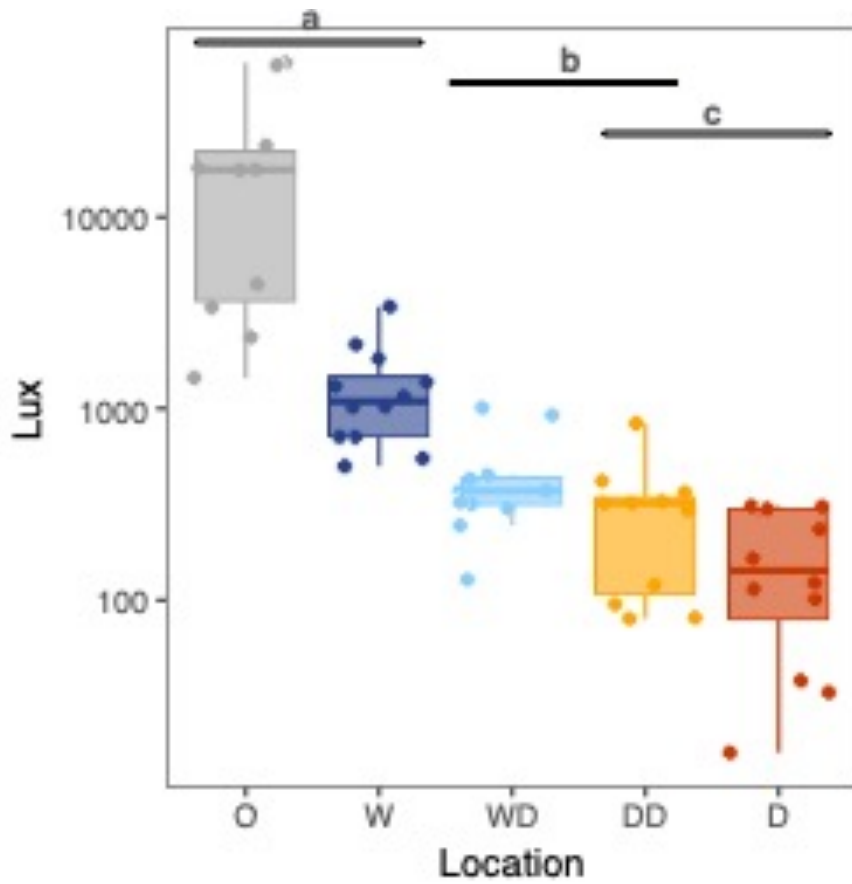


Figure S3. Relative abundance of sequence reads assigned to chloroplast 16S in each sample based on location inside, or outside (building exterior) of classrooms. “O” = outside window, “W” = inside window, “D” = inside door, “DD” = inside desk near door, “WD” = inside desk near window. Lowercase letters indicate statistically significant differences among samples.

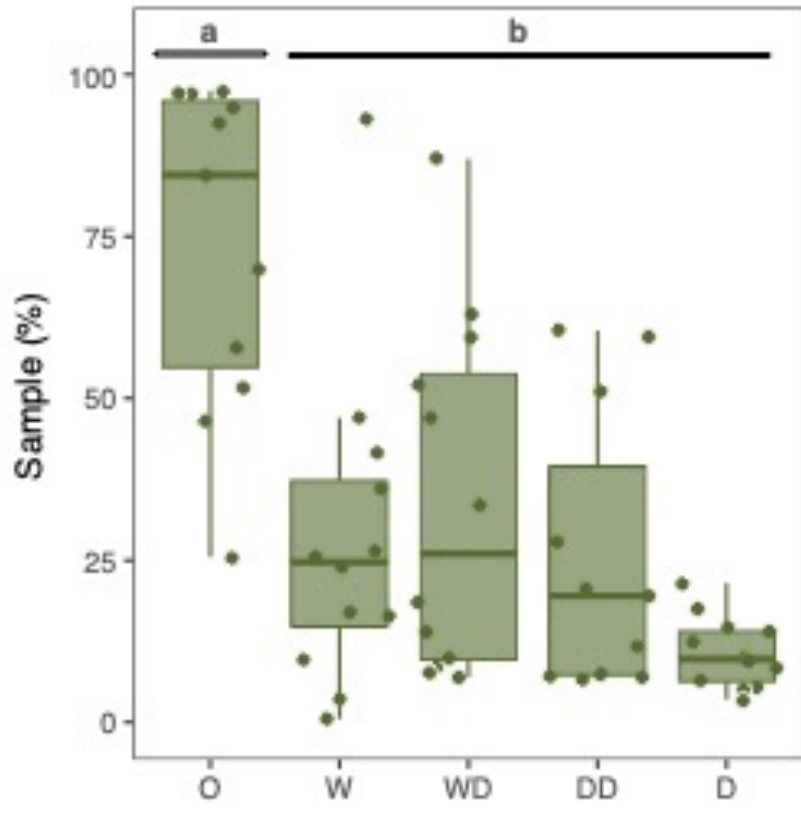


Figure S4: Bacterial communities of surfaces varied by inside/outside location, but not by inside location. NMDS ordination plot of the bacterial communities of inside samples as a function of location. Inside: Desk (D) is the desk nearest the inside classroom door, while Inside: Desk (W) is the desk nearest the window.

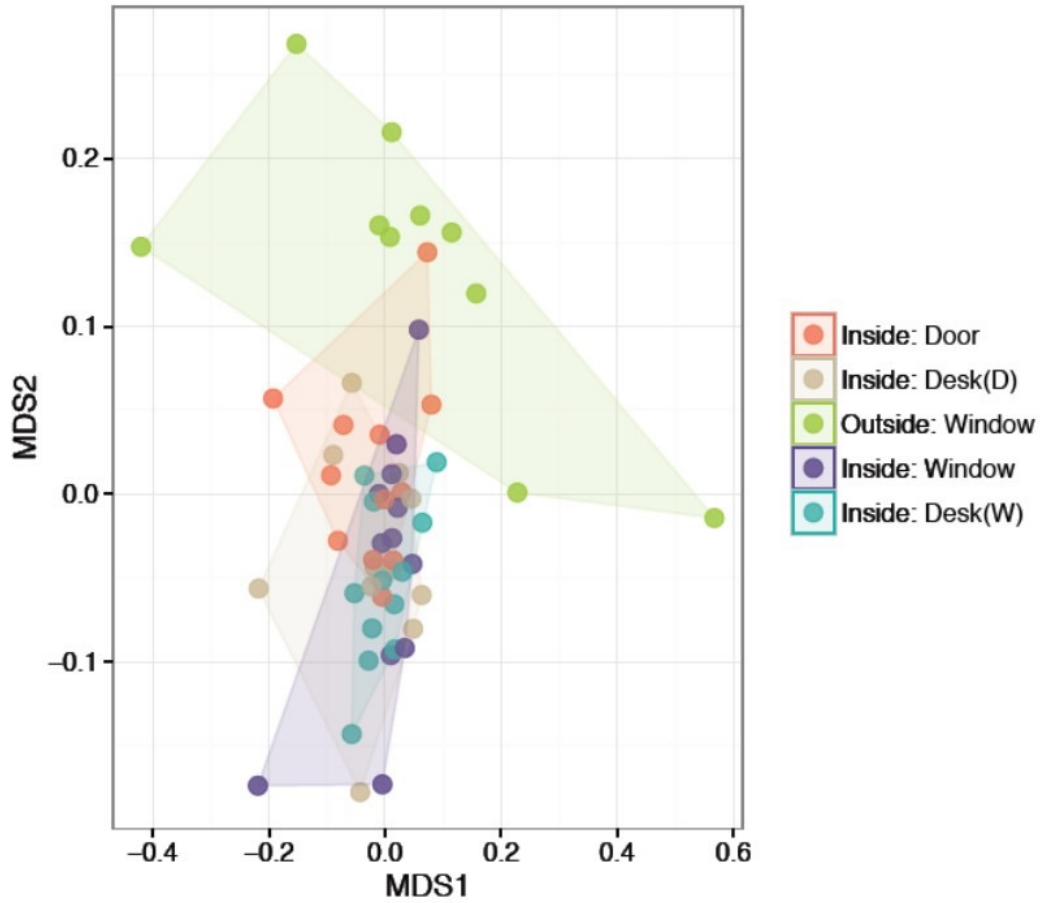


Figure S5. Heatmap of relative abundances of the 20 most abundant genera detected across sampled locations. “O” = outside window, “W” = inside window, “D” = inside door, “DD” = inside desk near door, “WD” = inside desk near window. Color scaled by genus.

	O	W	WD	DD	D
k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Nocardioideaceae; g__	1.2	1.8	2	2.3	1.2
k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Cytophagaceae; g__Hymenobacter	4.3	1.1	0.5	0.6	0.4
k__Bacteria; p__Cyanobacteria; c__Oscillatoriorhynchidae; o__Chroococcales; f__Xenococcaceae; g__	10	0.5	0.3	0.3	0.5
k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Staphylococcaceae; g__Staphylococcus	0	0.8	2.5	1.7	1.3
k__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus	0.1	7.7	9.7	6.6	4.7
k__Bacteria; p__Firmicutes; c__Clostridia; o__Clostridiales; f__Clostridiaceae; g__SMB53	6.7	0	0.1	0	6.2
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Cohaesibacteraceae; g__	6.6	0	0	0	0
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Methylobacteriaceae; g__Methylobacterium	4.4	2.2	1.2	1.5	2
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiales; f__Methylocystaceae; g__	7.6	0.9	0.5	0.3	0.7
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiales; f__Agrobacterium	0.9	2	1.7	1.2	1.7
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Rhizobiales; unclassified; unclassified	3.1	2.8	0.7	0.5	0.8
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__	2.2	2	1.7	1.2	2
k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter	1.4	1.8	1	0.8	1
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__	2.4	2.8	2.2	2.4	5.8
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__	2.4	1.2	0.7	1	0.5
k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Janthinobacterium	2.8	4.2	3.2	3.9	2.7
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Erwinia	0.3	1.8	1.8	0.8	2.5
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Trabulsiella	0.1	1.2	1.4	1	1.7
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae; g__Acinetobacter	0.3	6.3	10.7	8.5	3
k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas	4.7	6.2	6.2	10.2	7.9
Other	38.5	52.7	52	55.2	53.4

Figure S6. Differentially enriched OTUs in high contact surface communities (desks, n=19) versus low contact surface communities (door trim, n=11) across classrooms as determined by a DESeq2 negative binomial Wald test with FDR corrections. Twenty-three OTUs were significantly enriched across sample types. Notably, OTUs assigned to taxa frequently found in human saliva and skin (e.g., taxa in the families Streptococcaceae, Micrococcaceae, and Moraxellaceae) were enriched in bacterial communities on high contact surfaces.



Table S1.

List of putative contaminant OTUs found in the negative control samples that were removed from the original dataset.

OTU ID	taxonomy
OTU_5911	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Mycoplana; s__
OTU_209	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Mycoplana; s__
OTU_42	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Oxalobacteraceae; g__Janthinobacterium; s__lividum
OTU_91	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Moraxellaceae; g__Acinetobacter; s__
OTU_3565	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Asticcacaulis
OTU_3618	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__veronii
OTU_73	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__veronii
OTU_227	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__; s__
OTU_40	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__
OTU_1768	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Mycoplana; s__
OTU_4484	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas; s__veronii
OTU_53	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Caulobacterales; f__Caulobacteraceae; g__Mycoplana; s__
OTU_228	k__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Comamonadaceae; g__Methylibium; s__
OTU_1507	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae; g__; s__
OTU_2385	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Stenotrophomonas; s__
OTU_2061	k__Bacteria; p__Fusobacteria; c__Fusobacteriia; o__Fusobacteriales; f__Leptotrichiaceae; g__Sneathia; s__
OTU_5951	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Trabulsiella; s__
OTU_1951	k__Bacteria; p__[Thermi]; c__Deinococci; o__Deinococcales; f__Deinococcaceae; g__Deinococcus; s__
OTU_5294	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales
OTU_2287	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__Myxococcales; f__0319-6G20; g__; s__
OTU_2440	k__Bacteria; p__Cyanobacteria; c__4C0d-2; o__MLE1-12; f__; g__; s__

Table S2.

Table of statistical results at different sequencing depths per sample.

Statistical comparison	Sequencing depth	Total samples included in analysis	Test	Test statistic	p-value *
Inside vs outside window samples	200	22	Adonis	F=2.209, R ² =0.10	0.001
	1000	17	Adonis	F=2.6423, R ² =0.15	0.004
Inside samples by center and class. Factor: Center	200	47	Adonis	F=1.47, R ² =0.15	0.001
	1000	41	Adonis	F=1.91, R ² =0.21	0.001
Inside samples by center and class. Factor: Class	200	47	Adonis	F=1.16, R ² =0.14	0.005
	1000	41	Adonis	F=1.19, R ² =0.04	0.003
Desks versus door trim (High vs. low contact)	200	35	Adonis	F=1.43, R ² =0.04	0.005
	1000	30	Adonis	F=1.54, R ² =0.052	0.006
Inside by locations	200	47	Adonis		ns
	1000	41	Adonis		ns
Correlation between inside communities and lux	200	47	Mantel		ns
	1000	41	Mantel		ns
Correlation between inside communities and child density	200	47	Mantel	r=0.19	0.04
	1000	41	Mantel	r=0.251	0.022

* ns = not statistically significant

Table S3.

Mantel tests based on Pearson's product-moment correlation of Bray-Curtis distance metrics of lux and child occupant density for inside samples (inside window, desk by window, desk by door, inside door trim) across classrooms (samples: n=47):

Metric: bacterial beta diversity by classroom	p-value	Mantel statistic (r)
Occupant Density	0.04*	0.19
Metric: bacterial beta diversity by sampling location		
Lux	0.25	0.06