Assessing the temporal relationship between the environment of the neonatal intensive care unit and the early infant gut microbiome

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Introduction

Background

- The human gut is the largest and most diverse microbiome in the body
- The interactions between the gut microbiome and human host are instrumental in human health and disease
- The infant gut microbiome is more easily influenced by external factors such as those associated with the environment
- It is critical that the infant gut microbiome develops with the optimal distribution of microbes for healthy immune function
- Patients in the neonatal intensive care unit (NICU) are more likely to have gut dysbiosis and are susceptible to severe diseases like necrotizing enterocolitis
- Stool evaluation gives a comprehensive look into the makeup of an infant's gut microbiome.

Dataset

- 16S rDNA amplicon reads from 1,607 environmental and stool samples curated across 25 patients tracked over time in the NICU at Children's Hospital New Orleans LCMC Health
- Environmental samples came from various sites in the NICU:
  - Alarm cancel switch, computer enter key, floor, sink drain, light switch, erase board ledge, incubator portal, stethoscope
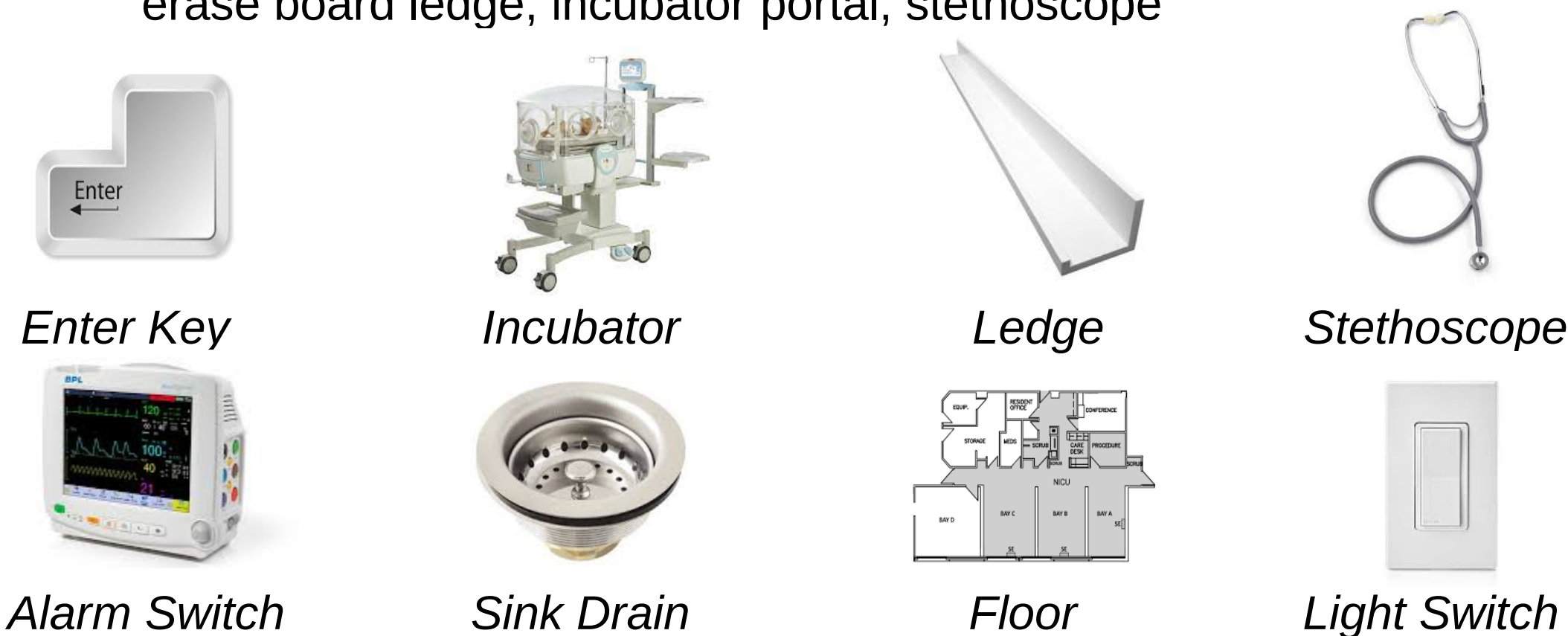
Enter Key   *Incubator*   *Ledge*   *Stethoscope*

Alarm Switch   *Sink Drain*   *Floor*   *Light Switch*

Figure 1. Environmental Sites in NICU

Research Aim

- To investigate the contributions of microbial communities in the NICU to the infant stool microbial communities

Hypothesis

- The NICU environment does have a temporal effect on the early infant gut microbiome

Methods

1. Amplicon reads were denoised for quality and amplicon sequence variants (ASVs) were inferred and taxonomically classified using the DADA2 pipeline in R
2. ASVs were decontaminated with mock community and negative control samples using the R package *decontam*
3. A phylogenetic tree was generated with the neighbor-joining tree estimate using the R package *phangorn*
4. Sample ordination was performed through multidimensional scaling with weighted Unifrac distance using the R package *phyloseq*
5. Alpha diversity was calculated and plotted using *phyloseq*
6. A Gibb's sampler implemented in the package *SourceTracker2* was used to determine the proportions of a stool microbial community that came from environmental sites of the NICU which was done for each stool sample with environmental samples taken within two days of the stool sample; this returned a time series of contributions each site made to stool samples across all patients
7. The time series were normalized using Gaussian process regression with the R package *GauPro*
8. The normalized time series were used to train a self-organizing map (SOM), a type of artificial neural network that is trained using unsupervised learning, using the R package *kohonen*
9. The SOM was partitioned into 4 clusters using hierarchical clustering with the R package *stats*
10. Dynamic Time Warping Barycenter Averaging was used to find the optimal average time series for each cluster with the R package *dtwclust*
11. Visualizations were generated using the R package *ggplot2*
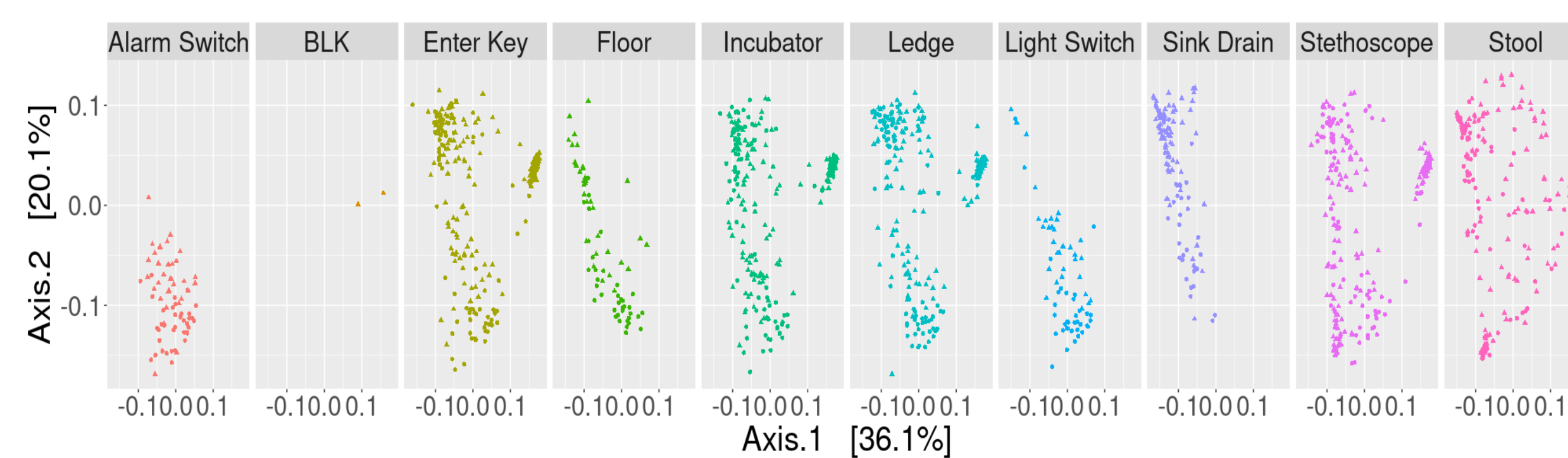
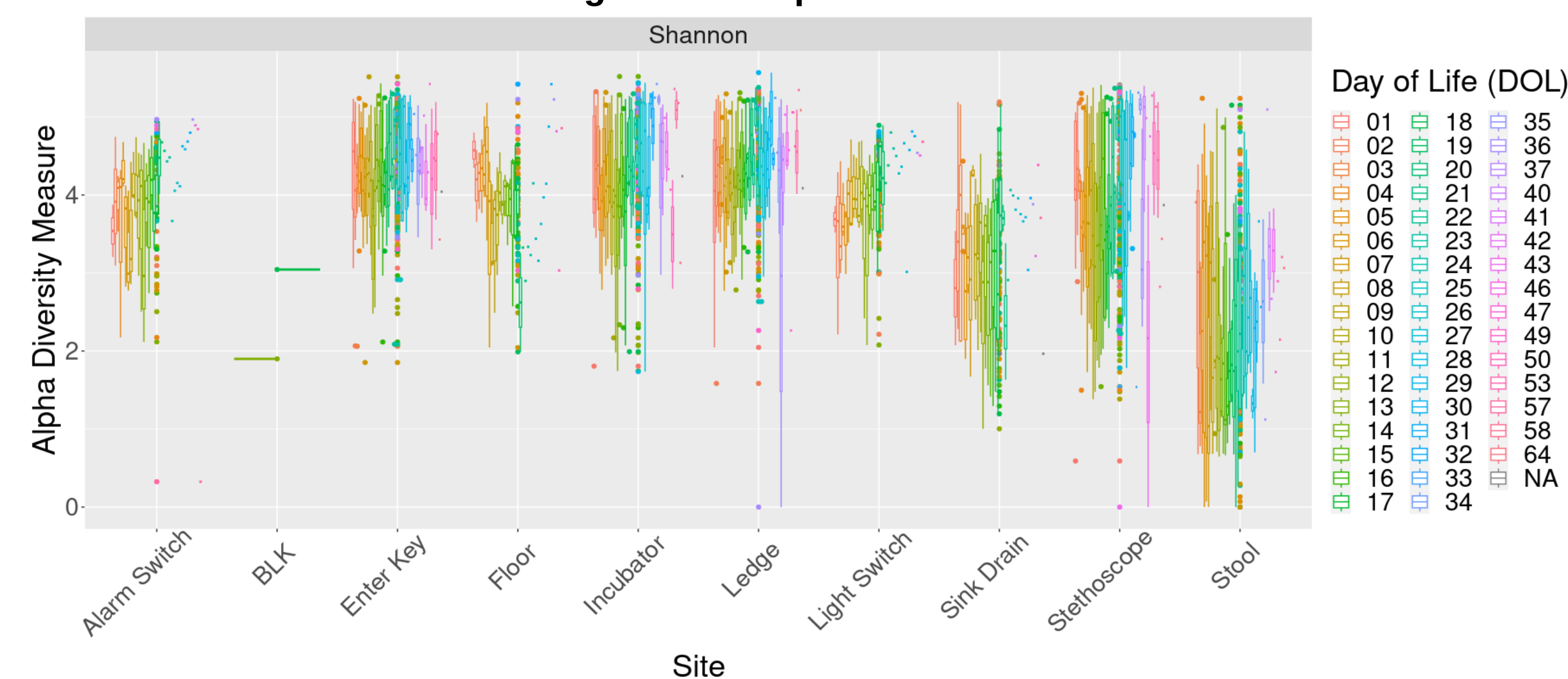## Microbial Communities Visualized

Figure 2. Sample Ordination

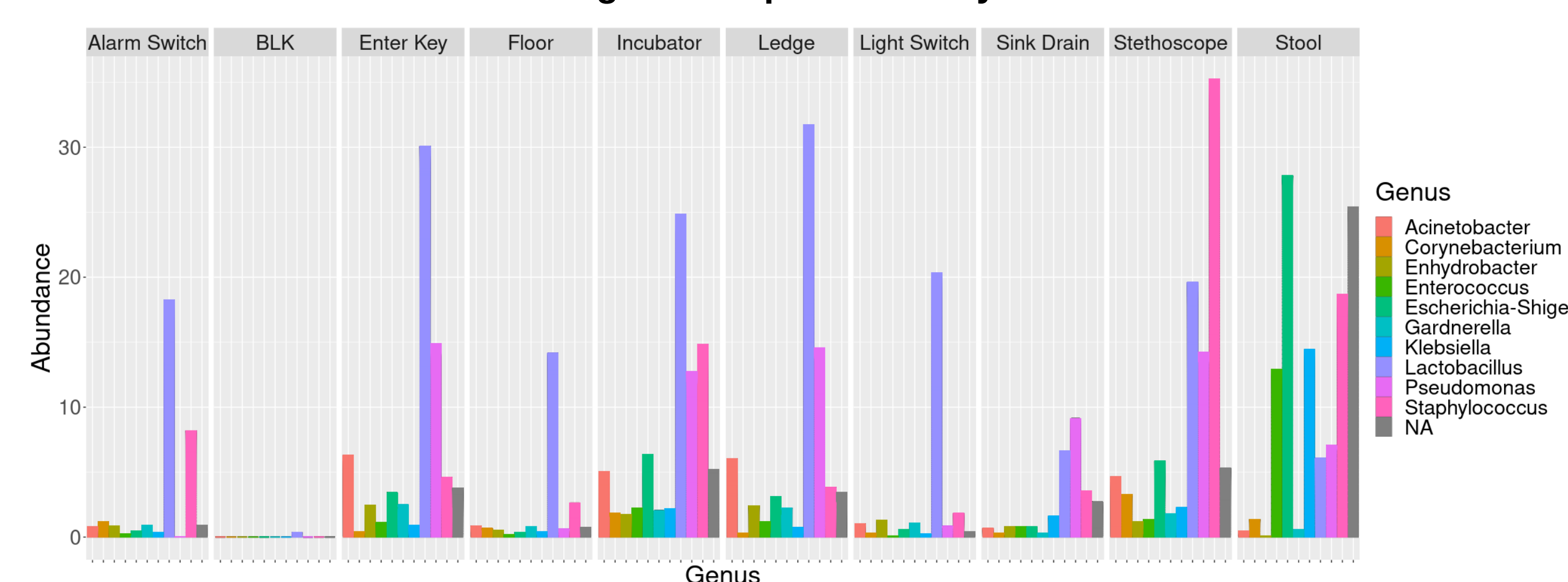**Figure 3. Alpha Diversity**

Figure 4. Relative Abundance of Most Abundant Taxa
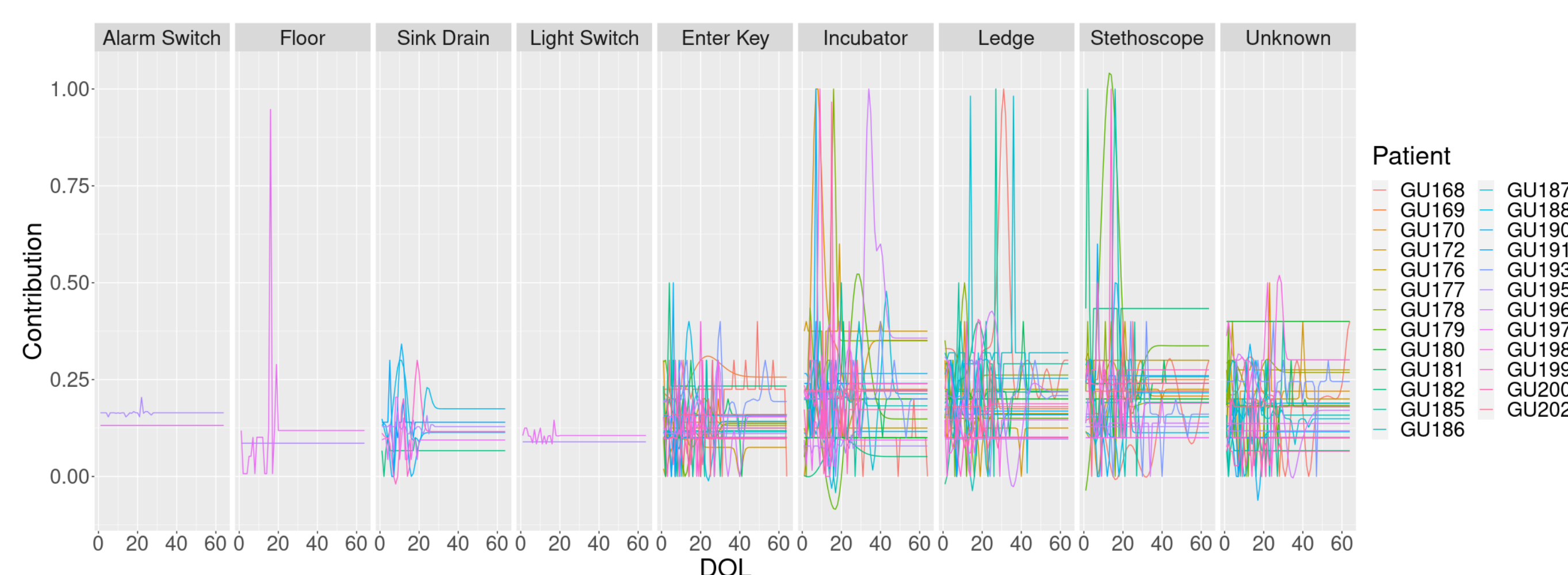
Microbial Source Tracking

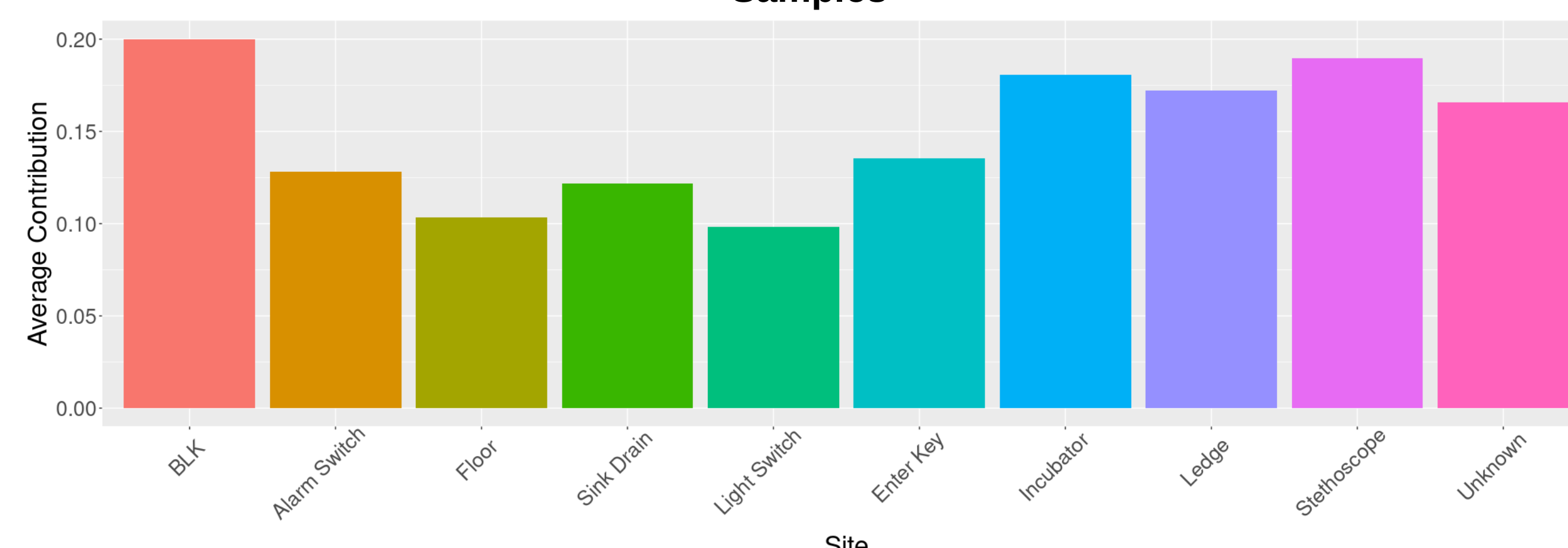**Figure 5. Normalized Time Series of NICU Environmental Contributions to Stool Samples**

Figure 6. Average Contribution of NICU Environmental Sites to Stool Samples
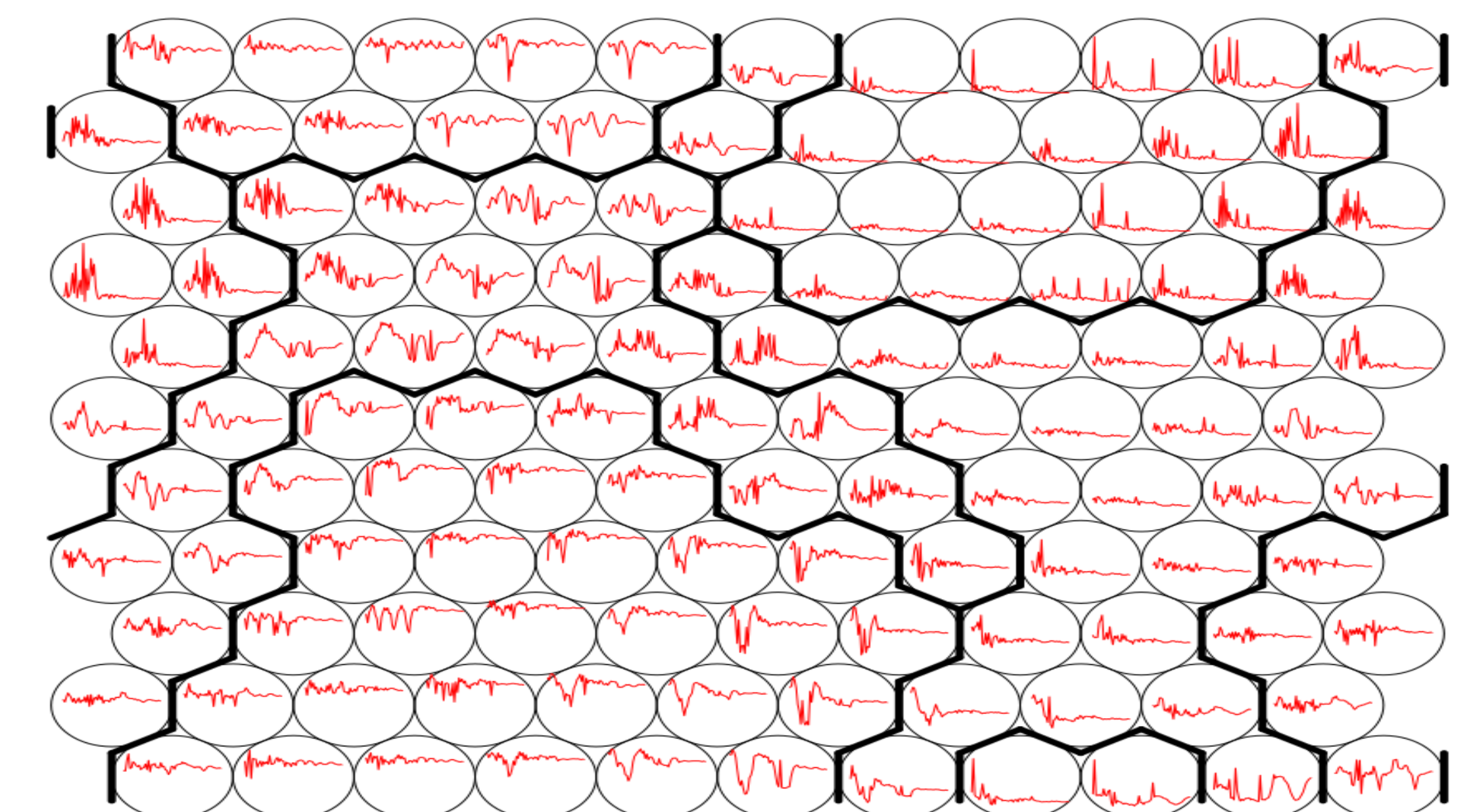
Time Series Clustering

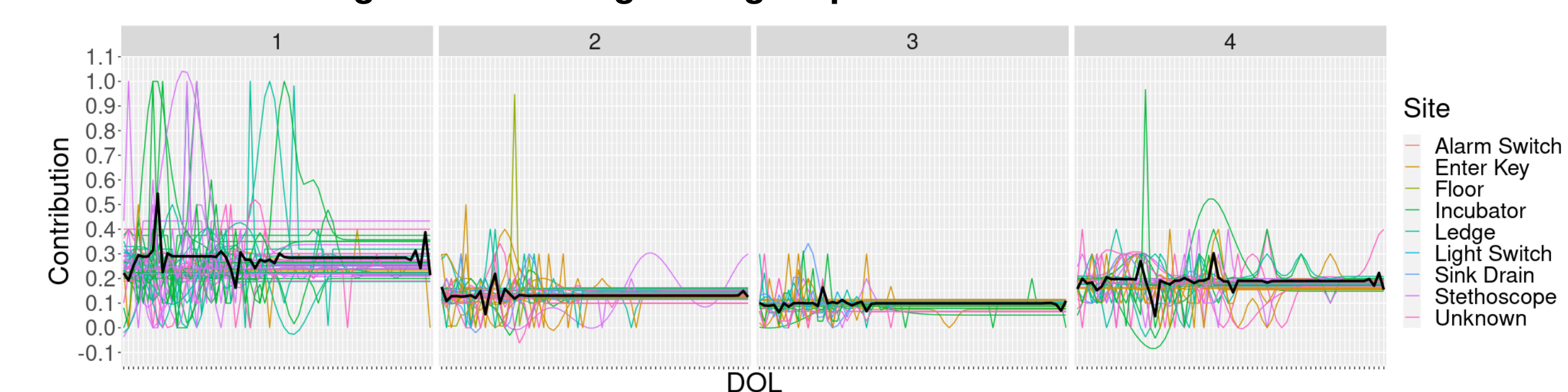**Figure 7. Self-Organizing Map with Cluster Boundaries**
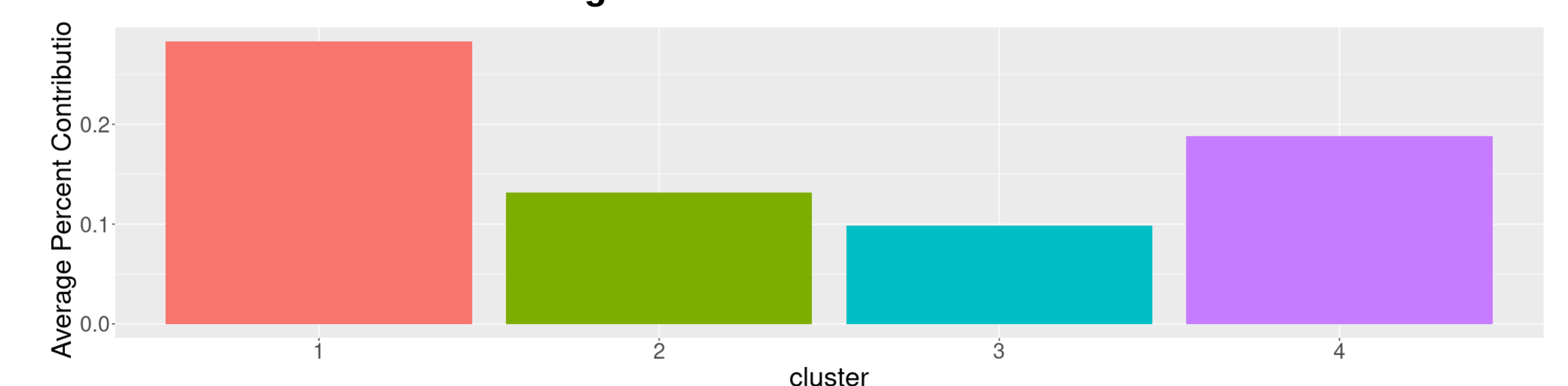
Figure 8. Time Series Clusters

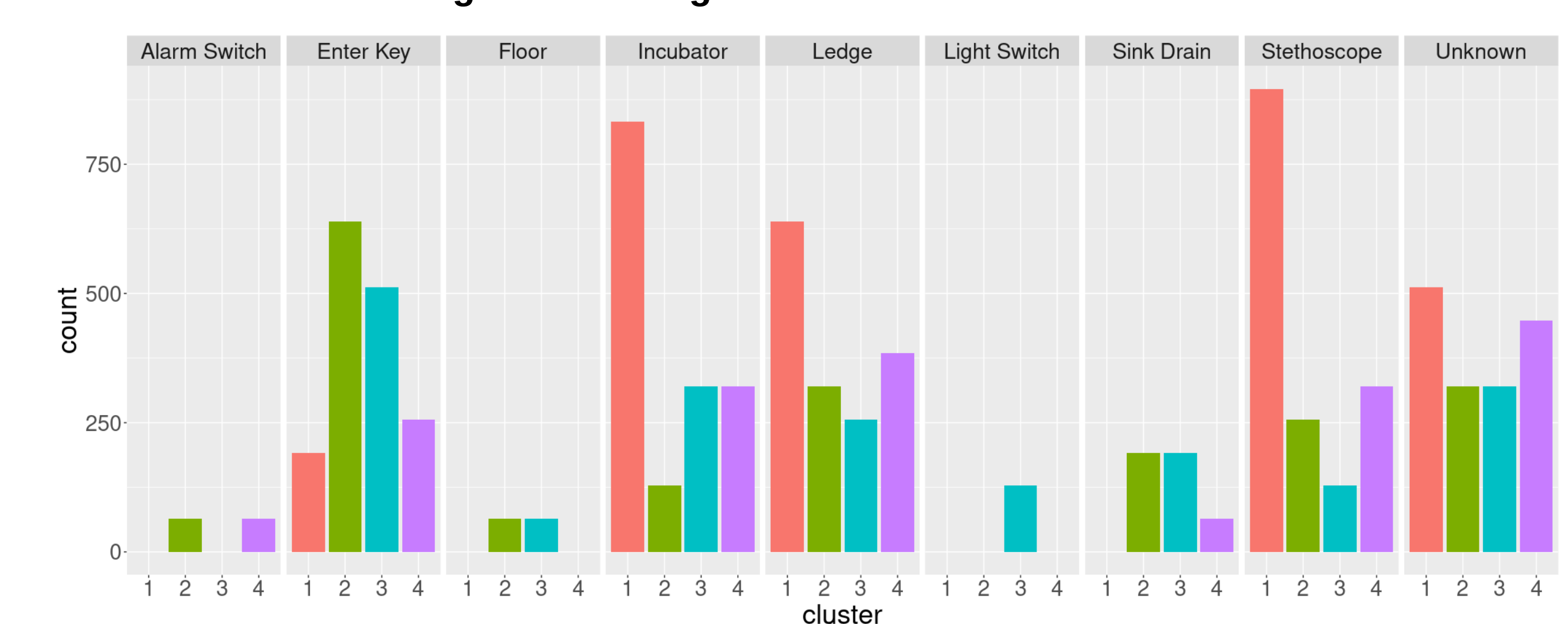**Figure 9. Average Contribution of Clusters**

Figure 10. Cluster Distribution of NICU Environmental Sites

Figure 11. Cluster Distribution of Patients

Conclusion

- The NICU environment does affect the infant gut microbiome
  - Environmental sites had a positive contribution to microbial communities in stool samples with Incubator, Ledge, Stethoscope, and BLK having the highest average contribution
- Different environmental sites of the NICU follow typical temporal trends with the infant gut microbiome
- The NICU can provide an important source of microbes that colonize the early infant gut