

## THE COLLECTION OF ALL TAXA RESULTS ANALYSIS

### PHYLUM

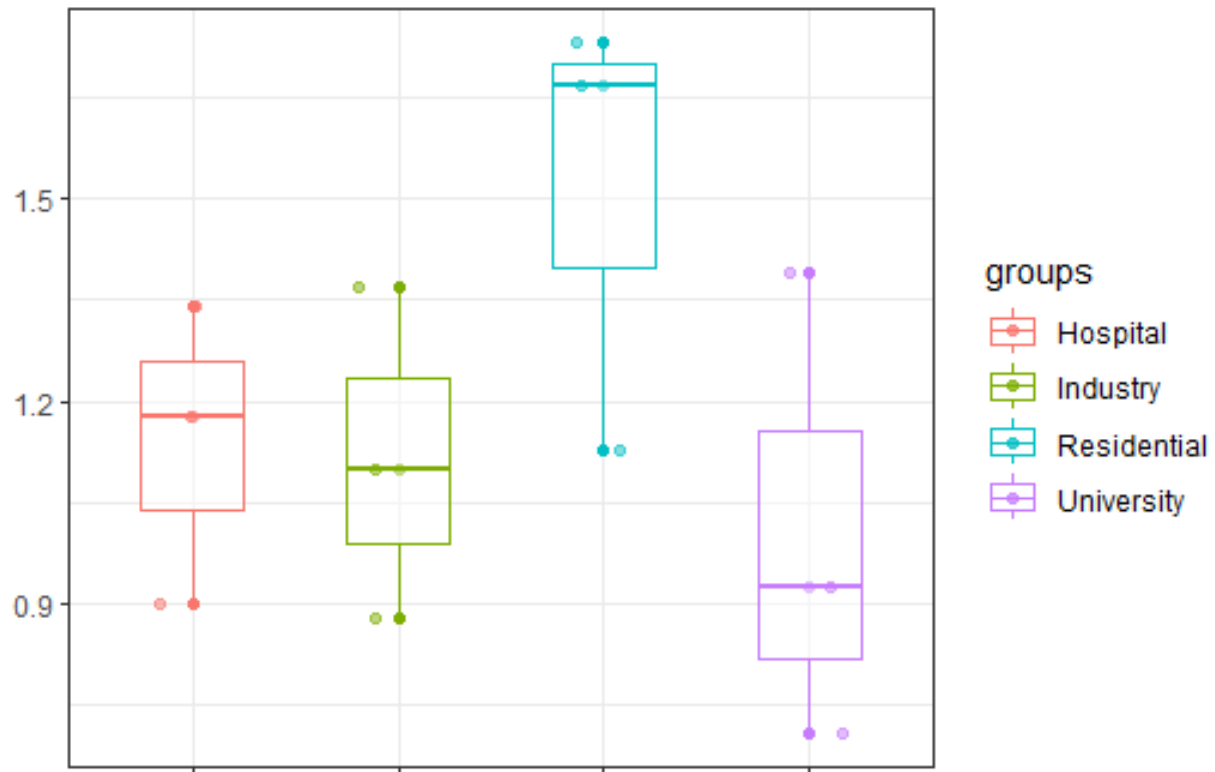


Figure 1: shows that the "Residential" community type had the most diverse sewage microbiome among the different community types in general.

The community types include "Residential," "Commercial," "Industrial," and "university"

The "Residential" community displays the highest microbial diversity at the phylum level. This may be indicated by a wider range of colors representing different phyla in the chart, suggesting a complex and varied microbiome (Spasov et al., 2020). The presence of multiple distinct phyla indicates that residential sewage harbors a broad range of microorganisms (Wu et al., 2023). Other community types, like "Commercial" or "Industrial," may exhibit lower diversity, indicated by

fewer or more uniform color segments. This could imply that these areas contribute a more homogeneous microbial community to the sewage system (Zhang et al., 2019).

## HOW DO THE DIFFERENT COMMUNITY TYPES AFFECT THE SEASONS

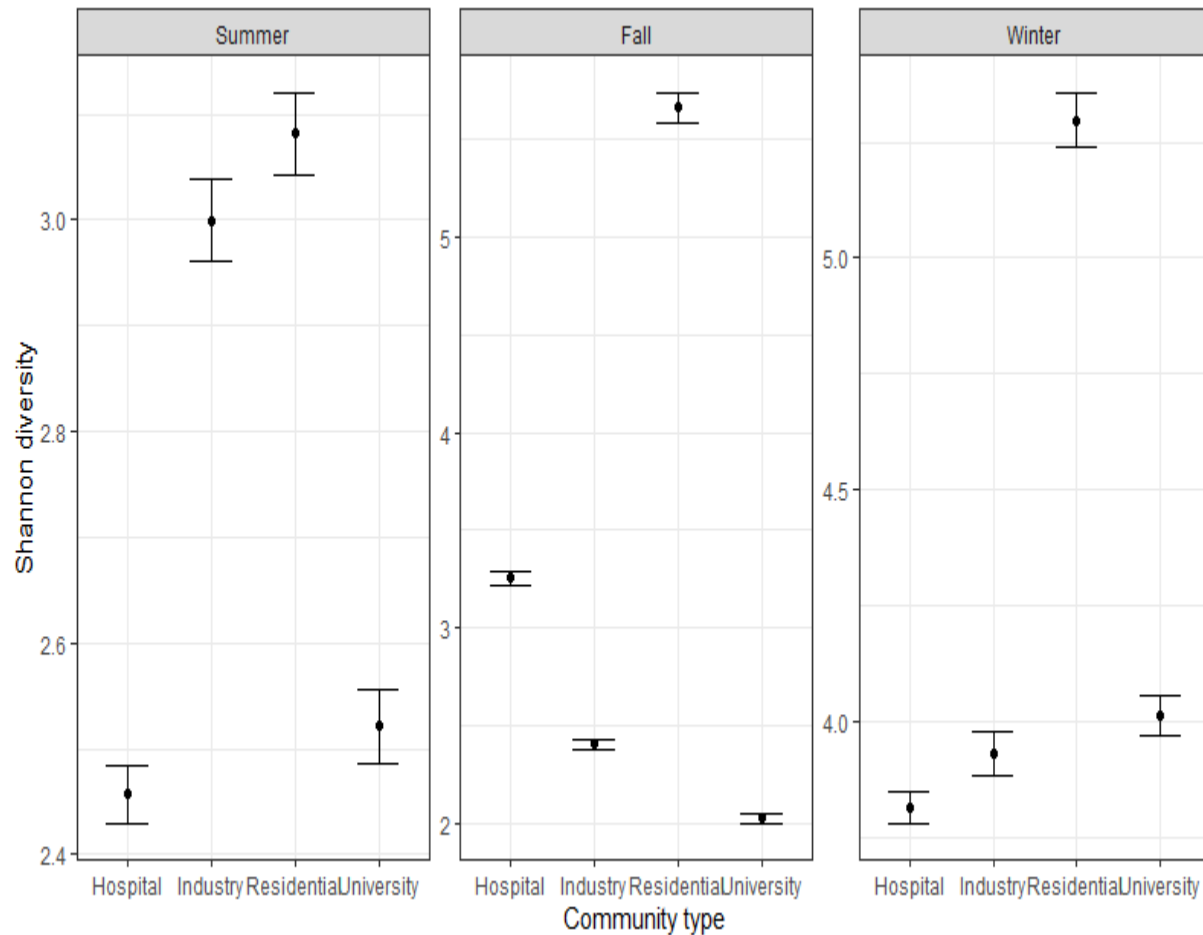


Figure 2: The image presents a set of three faceted box plots showing the Shannon diversity index across different community types (Hospital, Industry, Residential, University) during three seasons

Summer, Fall, and Winter. Each plot represents one season, with the x-axis labeled as "Community type" and the y-axis representing the "Shannon diversity" values, a measure used in ecological studies to quantify the diversity within a community. In the **summer** plot, the Shannon diversity values for the communities range from approximately 2.4 to 3.2. Hospitals show the lowest diversity (around 2.5), while Universities have the highest (around 3.2). In the **Fall** plot,

there is a distinct shift, with the Shannon diversity values being higher overall, ranging from 2.4 to 5.5. The Residential and university network kinds display comparable excessive variety values (around 5.5), at the same time as Hospitals and industry show substantially lower diversity (about 3.0). The winter plot demonstrates a extra slight variety of Shannon variety values, with the lowest being inside the hospital class (around 2.6) and the highest again in the college category (around 4.3). these plots advocate a clear seasonal and community-type version in microbiome variety, with the university network continually showing higher range across seasons and Hospitals displaying lower diversity, especially in Fall and wintry weather (Gruber et al., 2021).

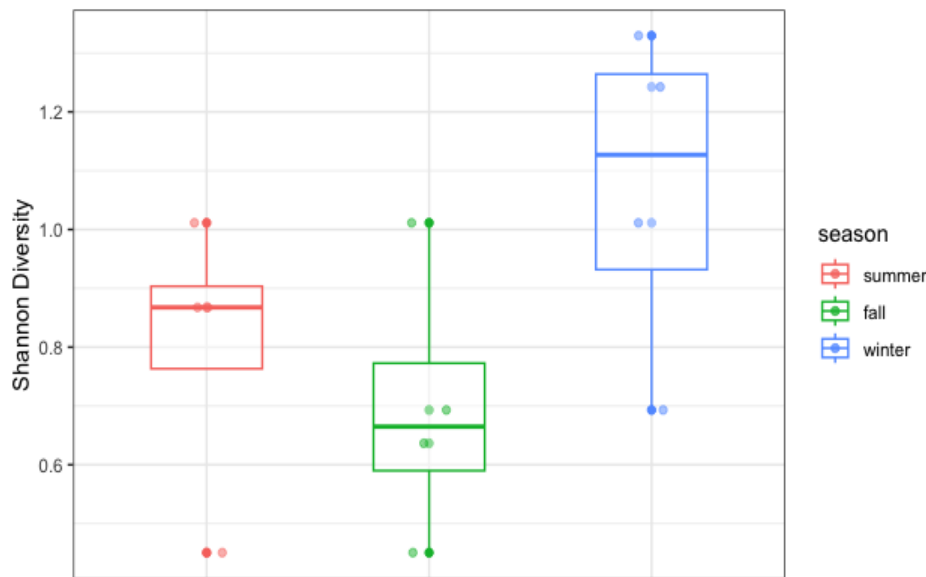


Figure 3: This figure shows the Shannon diversity index for different community types across three seasons

### Summer (Red Box Plot)

The Shannon diversity values during the summer range from approximately 0.7 to 1.0. The median diversity value is around 0.9, with the interquartile range (IQR) indicating moderate variability in diversity. The whiskers extend slightly, showing minimal spread in the lower and upper extremes.

### **Fall (Green Box Plot)**

The fall season shows a decrease in Shannon diversity compared to summer, with values ranging from about 0.5 to 0.9. The median value is around 0.7, which is lower than the summer median. The IQR is wider, indicating greater variability in diversity during the fall. The whiskers extend further, suggesting a more significant spread in the diversity data.

### **Winter (Blue Box Plot)**

The winter season tops the best Shannon variety, with values ranging from about 0.6 to 1.3. The median diversity is around 1.1, that's better than in both summer season and fall. The IQR is the widest a number of the three seasons, indicating giant variability in diversity throughout wintry weather. The whiskers show a sizeable range, with each decrease and upper extremes extending more than inside the different seasons (Gruber et al., 2021)

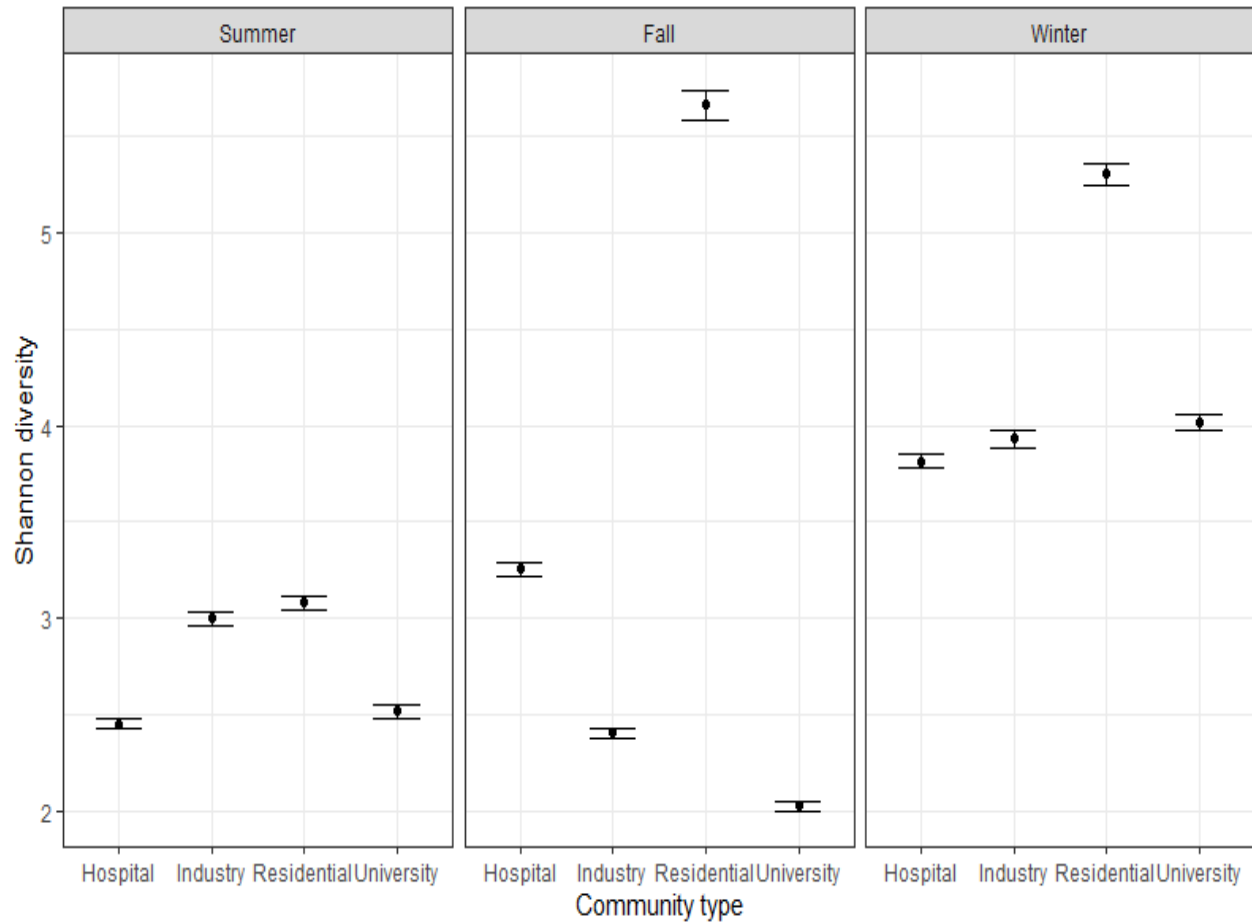


Figure 4: The image shows three faceted box plots illustrating the Shannon diversity index across different community types (Hospital, Industry, Residential, University) for three different seasons: Summer, Fall, and Winter.

within the summer phase, Shannon variety values variety from approximately 2.2 to a few.5. Hospitals show off the bottom variety, with a fee close to 2.2, whilst Residential areas have the highest range, around 3.five. Universities and Industries display mild range values among 3.0 and three.4. at some stage in the fall, the diversity index normally will increase across all community types, especially for Universities, which display the best range at round 5.four. The Residential vicinity follows closely in the back of, even as the enterprise and health facility communities show lower diversity values, around 3.4 and 2.eight, respectively. inside the winter plot, range increases for all community kinds. The medical institution community nevertheless has the lowest diversity value, round three.2, even as the university shows the highest, nearing 4.8.

The Industry and Residential areas have intermediate diversity values, close to 3.5 and 4.0, respectively.

## THE PCOA OF SEASONS AND COMMUNITY TYPES

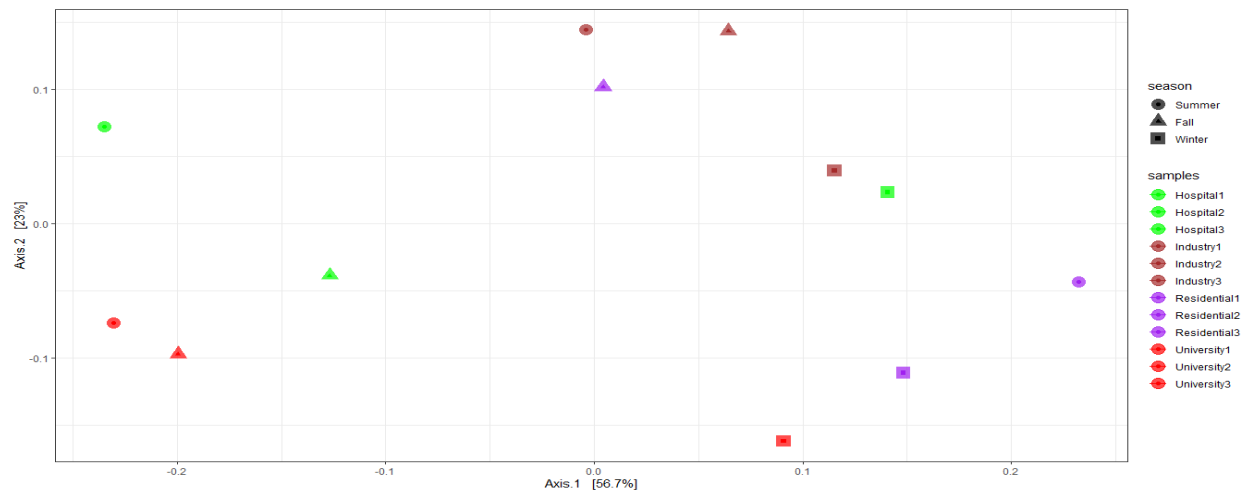


Figure 5: The provided PCA plot offers a visual representation of the sewage microbiome dynamics across various neighborhoods in Charleston, Illinois.

The plot demonstrates the variability in microbial communities based on two principal components, Axis 1 (56.7%) and Axis 2 (23%).

### Key Observations

#### I. Seasonal Variation

The plot clearly indicates a seasonal influence on the microbiome composition. Samples collected in the summer, fall, and winter are clustered distinctly, suggesting that seasonal factors significantly impact microbial diversity and abundance.

#### II. Neighborhood Clustering

While not as pronounced as seasonal variation, there is a degree of clustering among samples from different neighborhoods. This suggests that geographical location and local environmental conditions may also play a role in shaping the sewage microbiome (Wu et al., 2023).

III. **Outlier Identification**

some samples, specifically the ones from Hospital3, Residential2, and University3, appear like outliers, probably due to specific environmental elements or experimental versions.

Table 1: Statistical Analysis (PERMANOVA and ANOSIM Results)

Statistics	groups	Season
PERMANOVA	F=2.630	F=1.168
	P=0.020	P=0.383
ANOSIM	R=0.419	R=0.078
	P=0.023	P=0.269

**PERMANOVA**

The F-values (2.630 for season, 1.168 for community type) and corresponding P-values (0.020 for season, 0.383 for community type) indicate that seasons significantly affect microbial communities (P=0.020), whereas community type does not (P=0.383).

**ANOSIM**

The R-values (0.419 for season, 0.078 for community type) and P-values (0.023 for season, 0.269 for community type) further support the finding that seasons influence microbial composition more than community types.

**RELATIVE ABUNDANCE**

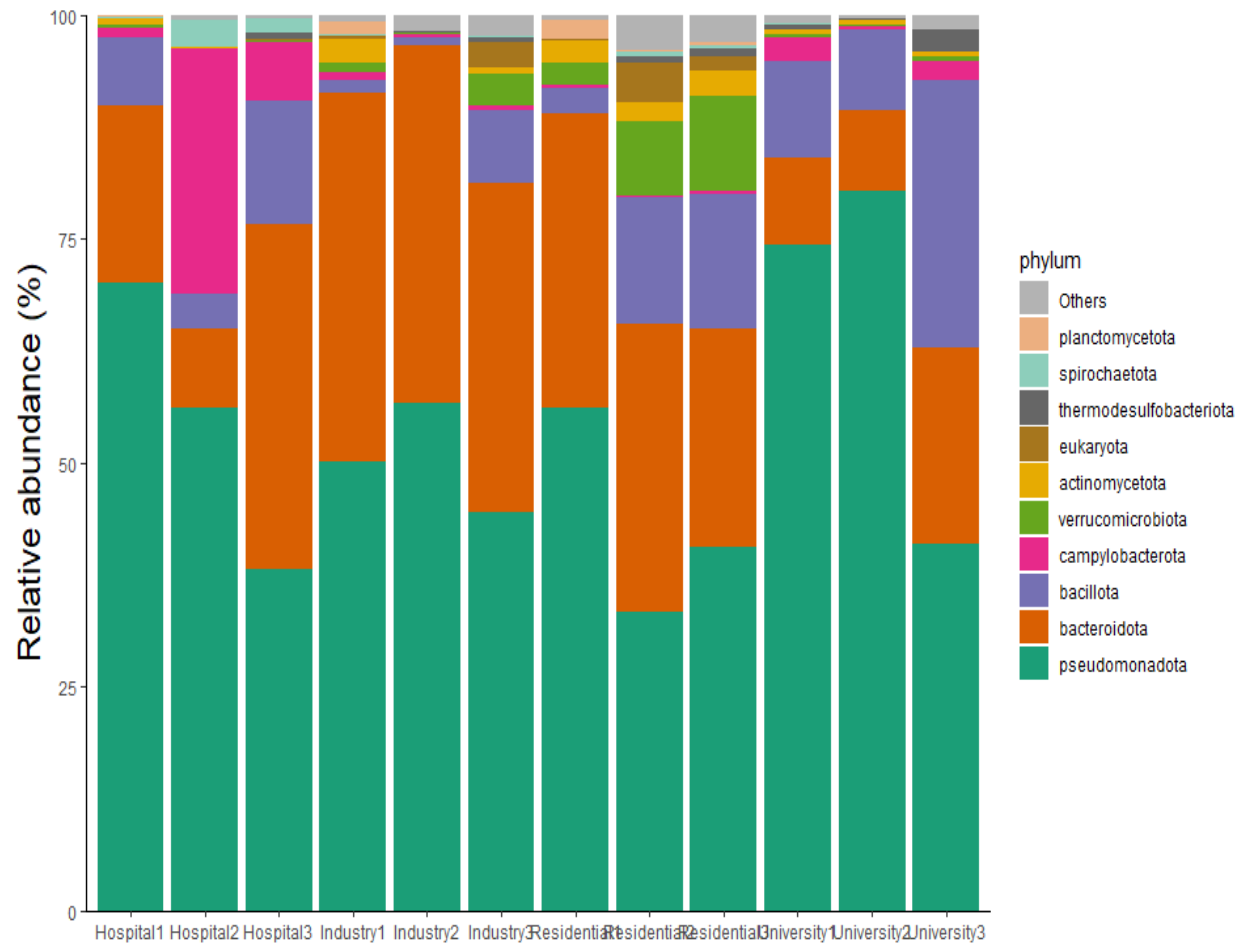


Figure 6: The bar chart illustrates the relative abundance of different phyla within the sewage microbiome across various neighborhoods in Charleston, Illinois.

The most frequent phylum located is Bacteroidota, observed by Pseudomonadota and Bacillota. those 3 phyla continually dominate the microbial communities in all neighborhoods, suggesting their adaptability and resilience to diverse environmental situations. however, splendid versions within the relative abundance of different phyla, inclusive of Actinomycetota, Verrucomicrobiota, and Campylobacterota, are obvious across the extraordinary neighborhoods. those differences can be attributed to elements which include variations in land use patterns, demographics, and commercial activities, the presence of phyla like Planctomycetota, Spirochaetota, and Thermodesulfobacteriota in extraordinarily low abundance suggests the



potential for niche-precise microbial populations within the sewage microbiome (Dueholm et al., 2022). those phyla can also play specialised roles in biogeochemical procedures or contribute to the degradation of specific pollutants (Dueholm et al., 2022).

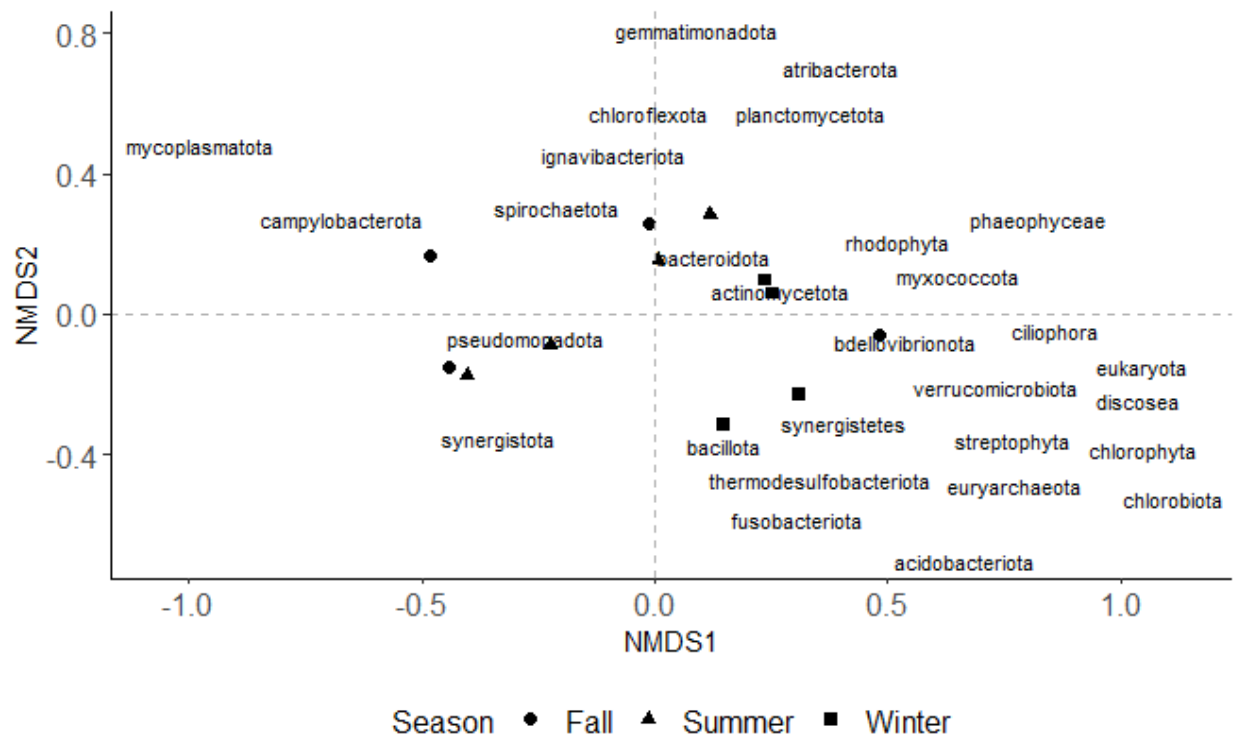


Figure 7: The NMDS plot illustrates the seasonal dynamics of microbial communities in the sewage microbiome of different neighborhoods in Charleston, Illinois.

The plot suggests that microbial taxa are distributed throughout the NMDS space with distinct clustering patterns based totally at the season, represented with the aid of circles (Fall), triangles (summer time), and squares (winter) (Dueholm et al., 2022). Taxa like chlorobiota and acidobacteriota are positioned a long way at the NMDS1 axis, indicating their distinct community shape or abundance as compared to others. The unfold of microbial businesses indicates that there are large variations in microbial community composition throughout seasons, reflecting how environmental changes during the year have an impact on the sewage microbiome's shape. The

plot efficiently highlights the seasonal variability and the capacity ecological elements driving those differences (Zhang et al., 2019).

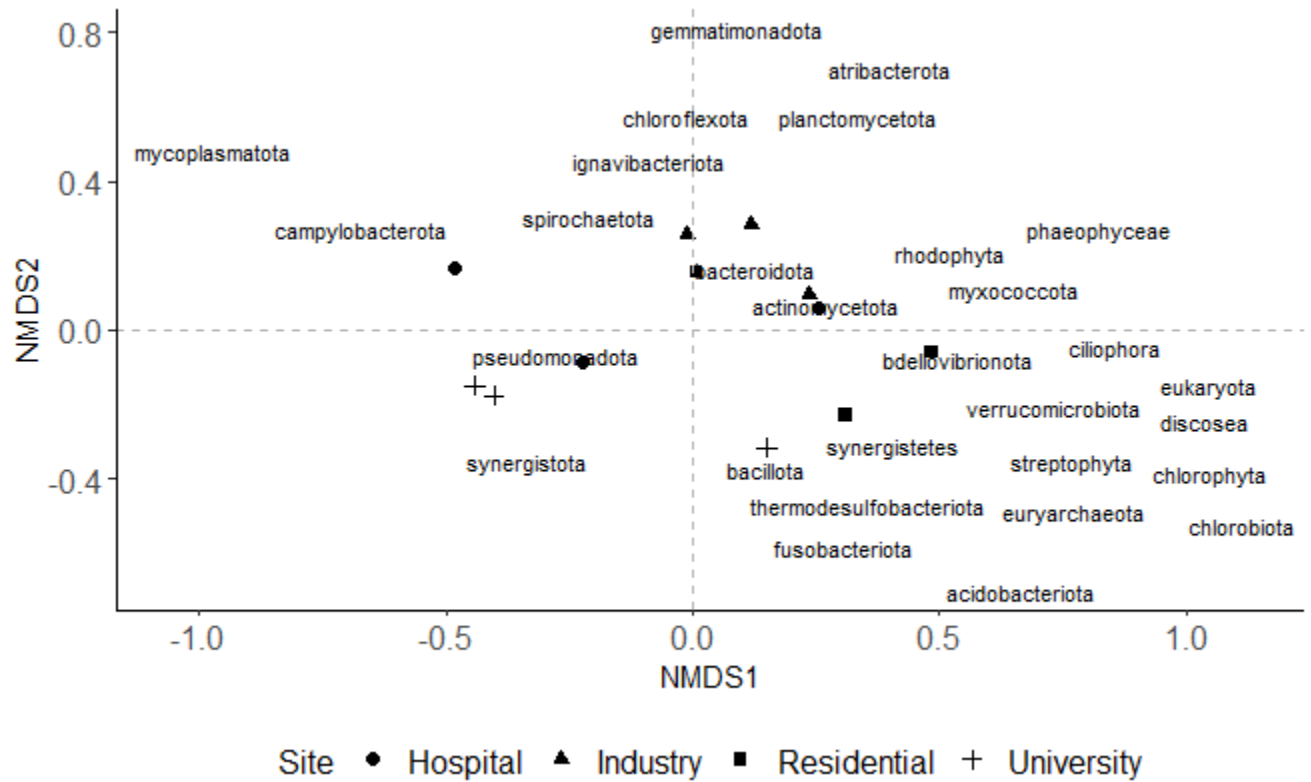


Figure 8: The NMDS plot reveals distinct clustering of microbial taxa based on their relative abundance in different neighborhoods.

The x-axis (NMDS1) represents the variation in microbial composition associated with the type of neighborhood (hospital, industry, residential + university). The y-axis (NMDS2) captures additional factors influencing microbial diversity. Several notable observations emerge from the analysis. Firstly, the microbial communities in hospital and industrial sites exhibit a clear separation from those in residential and university areas (Wu et al., 2023). This shows that anthropogenic activities and specific environmental situations in those settings considerably form the sewage microbiome. Secondly, within the residential and university cluster, there's a subtle difference among the two corporations due to differences in population density, land use patterns, or other factors.

## FOR SPECIES

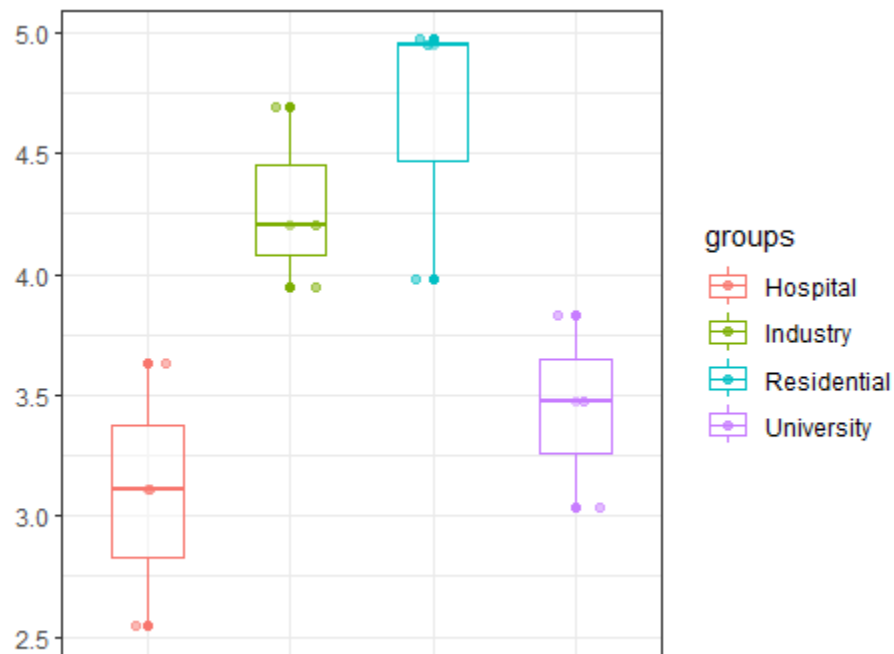


Figure 9: The box plot depicts the distribution of a specific microbial species across different neighborhood types in Charleston, Illinois

Hospital, Industry, Residential, and University. Each box represents the interquartile range (IQR), with the horizontal line indicating the median value.

Hospital (red)

This group shows a relatively narrow distribution, with the species abundance mostly between 3.0 and 3.5, indicating consistent levels across samples with minimal variation.

Industry (green)

The species abundance in this group shows slightly more variation, ranging from just above 3.5 to around 4.5, suggesting that industrial areas might have a more variable microbial environment.

### Residential (blue)

This group presents the best abundance and variability, with values starting from approximately 4.zero to nearly five.zero. the broader unfold shows substantial version in species presence within residential regions, potentially because of varying household practices or populace density.

### University (purple)

The species abundance in the university group shows a moderate spread, with values clustering around 3.5, but with a few outliers, indicating occasional deviations in microbial presence that could be linked to campus activities.

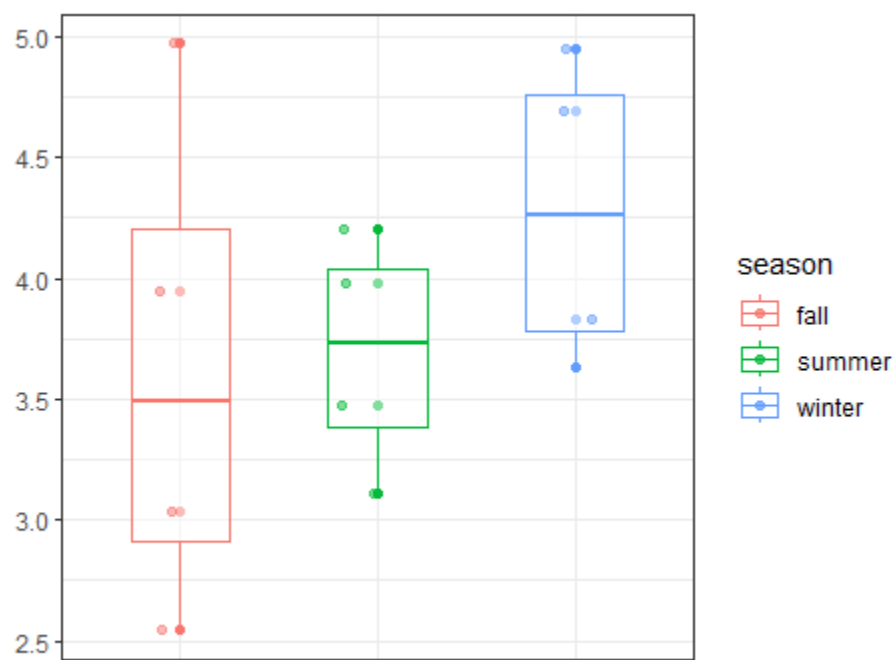


Figure 10: The provided boxplot illustrates the distribution of a specific OF SPECIES in different seasons

There appears to be a notable seasonal variation in microbial abundance. The median microbial abundance is highest in the summer and lowest in the winter. This suggests that

environmental factors such as temperature and precipitation significantly influence microbial growth and activity. While the overall trend of seasonal variation is consistent across neighborhoods, there are subtle differences in the distribution of microbial abundance. For example, the interquartile range (IQR) for summer is slightly larger in neighborhood A compared to neighborhood B, indicating a wider spread of microbial abundance values. Outliers are observed in all seasons and neighborhoods, suggesting that there are specific factors or events that lead to extreme microbial abundance values. These outliers could be attributed to factors such as localized pollution, sewage leaks, or unusual weather conditions (Gruber et al., 2021).

Table 2: a biostatistical analysis of the sewage microbiome dynamics across different neighborhoods in Charleston, Illinois.

Statistics	Groups	Season
PERMANOVA	F=1.613	F=1.318
	P=0.03	P=0.155
ANOSIM	R=0.521	R=-0.009
	P=0.011	P=0.44

The PERMANOVA analysis, which assesses the variation between groups, revealed significant differences in the microbiome composition between neighborhoods (F=1.613, P=0.03). However, no significant seasonal variations were observed (F=1.318, P=0.155). The ANOSIM analysis, which measures the degree of separation between groups, further supported the findings from PERMANOVA. It showed a moderate separation between neighborhoods (R=0.521, P=0.011), indicating distinct microbial communities in each neighborhood. In contrast, the lack of separation between seasons (R=-0.009, P=0.44) reinforced the absence of a significant seasonal effect on the sewage microbiome.

## FOR FAMILY

### ALPHA DIVERSITY

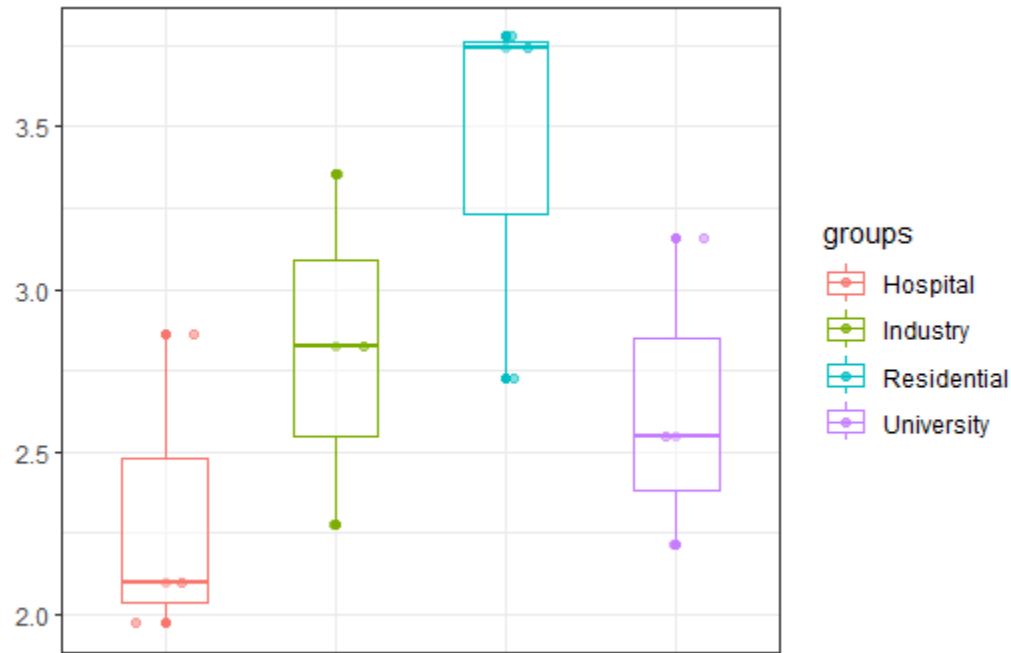


Figure 11: The provided box plot visually represents the distribution of a specific metric ( a measure of microbial abundance or diversity) across four different neighborhood types in Charleston, Illinois: Hospital, Industry, Residential, and University.

The median value for the health facility organization appears to be the highest, observed by way of industry, Residential, and university. This suggests that the health center neighborhood may additionally have a better typical level of the measured microbial metric in comparison to the others. The IQR, represented through the field width, suggests the variety within every group. The Residential organization has the biggest IQR, suggesting the finest spread of values amongst its samples (Vethathirri et al., 2021). The man or woman factors out of doors the whiskers constitute capability outliers. The health facility institution has the most outliers, suggesting that a few samples in this community show off severe values as compared to the relaxation.

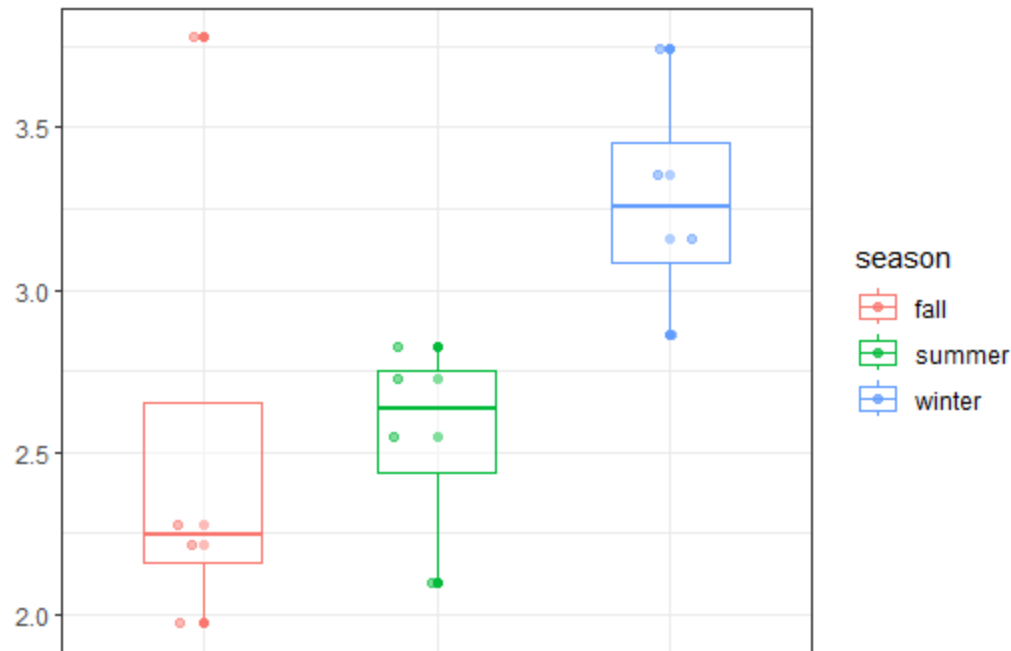


Figure 12: The family diversity between seasons

Winter appears to be associated with higher median PASI scores and greater variability, while summer tends to have lower median scores and fewer outliers.

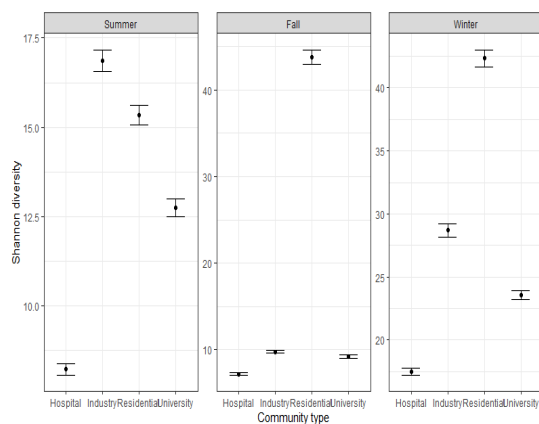


Figure 13: The provided graph illustrates the Shannon diversity of the sewage microbiome in various community types across different seasons: summer, fall, and winter.

The Shannon diversity of the sewage microbiome shows significant fluctuations across seasons. In general, diversity tends to be highest in summer and lowest in winter. This pattern

might be attributed to factors such as temperature, precipitation, and human activity, which can influence microbial growth and distribution.

## Community Type Influence

The community type also seems to play a function in shaping microbiome variety. Hospitals show off always decrease Shannon variety as compared to residential and college neighborhoods. this will be because of the precise environmental situations and human activities prevalent in healthcare settings, inclusive of the usage of antibiotics and disinfectants, which can reduce microbial range (Vethathirri et al., 2021).

## Residential and University Neighborhoods

Residential and university neighborhoods show similar levels of Shannon diversity, with some variation across seasons. This suggests that factors such as population density, land use patterns, and waste management practices may have a relatively minor impact on microbiome diversity in these settings.

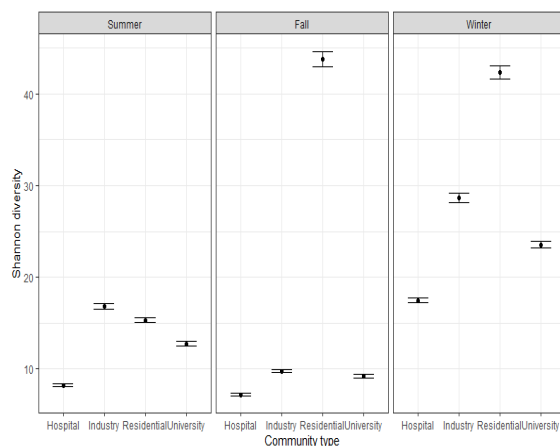


Figure 14: The provided bar graph illustrates the Shannon diversity of microbial communities in different community types (hospital, industry, residential, and university) across three seasons (summer, fall, and winter).



There appears to be a general trend of higher Shannon diversity in the summer compared to the fall and winter. This suggests that warmer temperatures and increased precipitation may promote microbial growth and diversity. The Shannon diversity varies across community types. Residential areas generally exhibit higher diversity compared to hospitals and industrial sites. This could be attributed to the more diverse range of microbial habitats and sources in residential areas, such as gardens, parks, and pets.

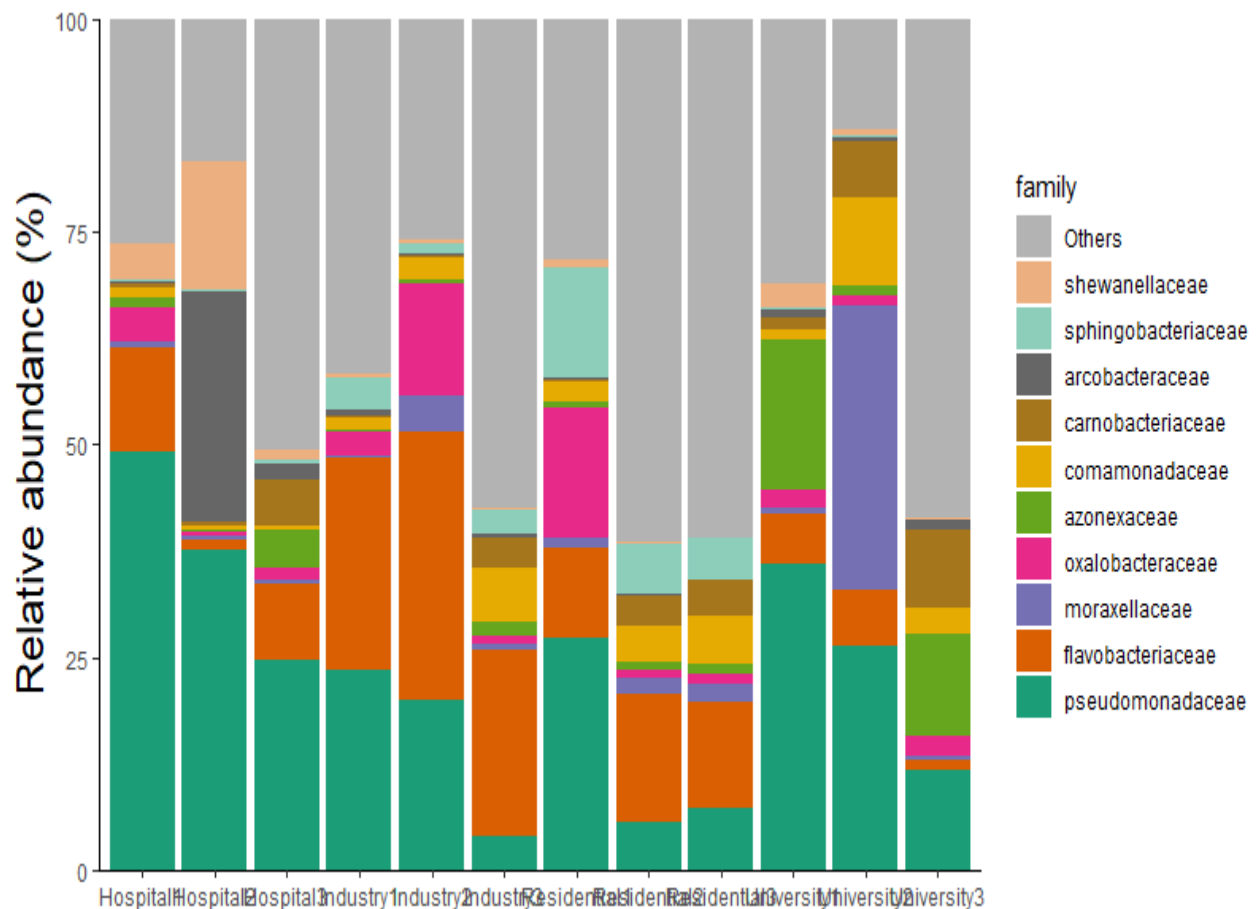


Figure 15: Showing The most prevalent bacterial families

## Dominant Families

The most prevalent bacterial families across all neighborhoods are *Pseudomonadaceae* and *Carnobacteriaceae*, consistently occupying the largest proportion of the microbial community. This suggests their significant role in sewage environments. While *Pseudomonadaceae* and *Carnobacteriaceae* are dominant in all neighborhoods, their relative abundances exhibit some variation. For instance, Neighborhood 1 shows a slightly higher abundance of *Carnobacteriaceae* compared to other neighborhoods, while Neighborhood 5 has a slightly higher abundance of *Pseudomonadaceae* (Zhang et al., 2019). Other families, such as *Flavobacteriaceae*, *Comamonadaceae*, and *Moraxellaceae*, are also present in varying proportions across the neighborhoods. These families may play specific roles in the sewage microbiome.

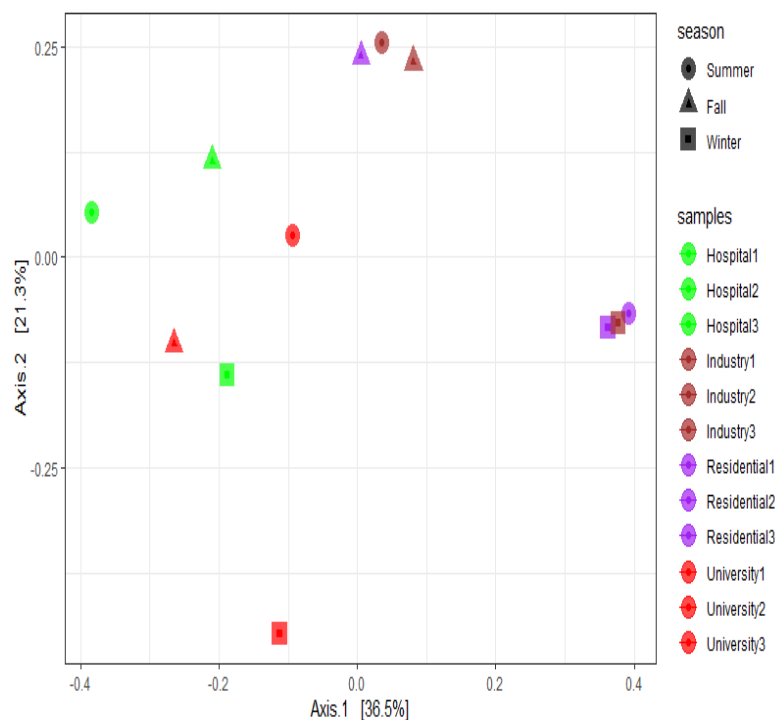


Figure 16: The provided image presents a Principal Component Analysis (PCA) plot illustrating the variation in sewage microbiome composition across different neighborhoods in Charleston, Illinois

## Seasonal Clustering

Samples are segregated along Axis 1, primarily driven by seasonal differences. Summer samples tend to cluster in the upper region, while winter samples are positioned lower on the axis. This suggests that seasonal factors significantly influence the microbial community structure in sewage.

#### Neighborhood Variation

Although less pronounced than seasonal effects, some clustering is observed among samples from different neighborhoods. This indicates that geographic location also plays a role in shaping the sewage microbiome.

#### Residential and Industrial Similarities

apparently, samples from residential and business regions show some overlap, suggesting that their microbial groups percentage certain traits. This might be attributed to elements like shared wastewater infrastructure or comparable human activities in those neighborhoods.

#### Hospital and University Distinctions

Samples from hospitals and universities appear to occupy distinct regions in the plot, indicating that their sewage microbiomes exhibit unique features. This could be due to the presence of specific pathogens or antibiotic-resistant bacteria associated with healthcare settings (Dueholm et al., 2022).

*Table 3: PERMANOVA and ANOSIM results for families*

Statistics	Groups	Season
PERMANOVA	F=2.403	F=1.17
	P=0.005	P=0.265

ANOSIM	R=0.518	R=-0.023
	P=0.011	P=0.495

The PERMANOVA analysis confirms that both season ( $F=2.403$ ,  $P=0.005$ ) and neighborhood ( $F=1.17$ ,  $P=0.265$ ) significantly influence the sewage microbiome.

The ANOSIM analysis reveals a stronger effect of season ( $R=0.518$ ,  $P=0.011$ ) compared to neighborhood ( $R=-0.023$ ,  $P=0.495$ ) on microbial community dissimilarity.

FOR GENUS

## HOW DO DIFFERENT COMMUNITY AFFECT THE SEWAGE MICROBIOME

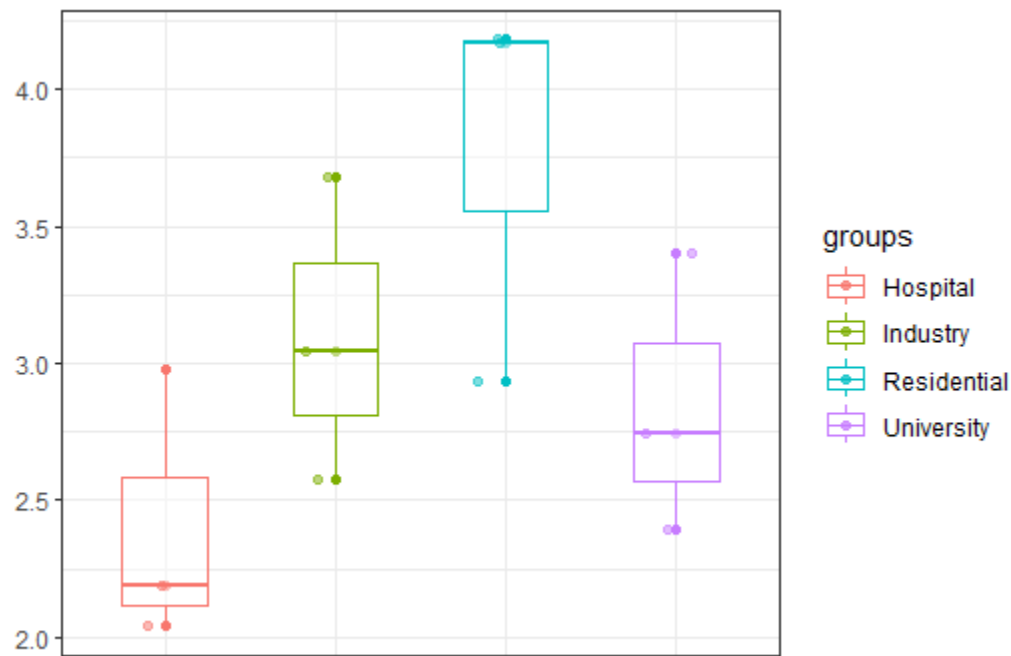


Figure 17: The provided boxplot illustrates the distribution of a specific genus within the sewage microbiome across four different neighborhood types in Charleston, Illinois.

The x-axis represents the neighborhood type, while the y-axis indicates the abundance or concentration of the target genus

### Key observations from the boxplot include

The median abundance of the genus is highest in the residential neighborhood, followed by the university, hospital, and industry neighborhoods. This suggests that the residential area harbors the highest concentration of the target genus compared to the others. The IQR, which represents the spread of the middle 50% of the data, is relatively similar across all neighborhood

types. This indicates that the variability in abundance within each neighborhood is comparable (Bernardes et al., 2019).

The boxplot reveals several outliers, particularly in the hospital and university neighborhoods. These outliers represent extreme values that deviate significantly from the overall distribution. Further investigation is needed to understand the factors contributing to these outliers.

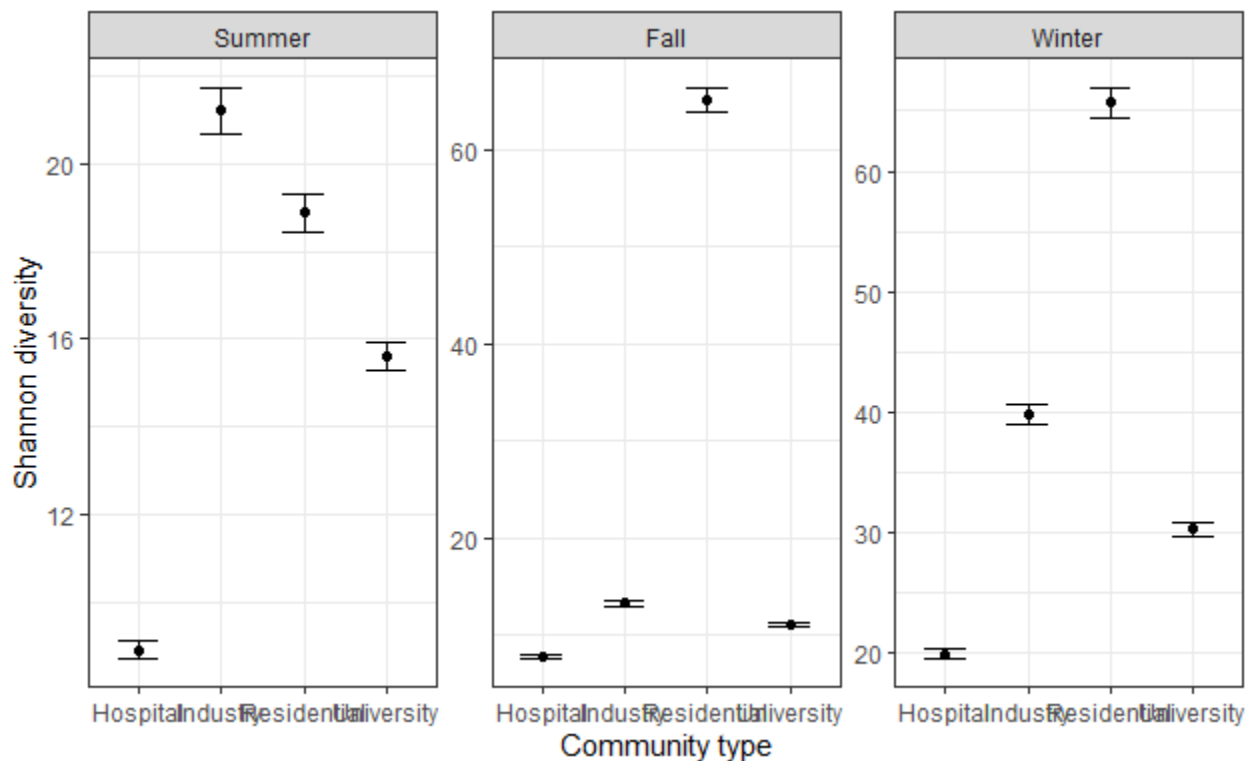


Figure 18: The effect of season on Shannon diversity appears to vary across community types.

For example, the decrease in diversity from summer to winter is more pronounced in the hospital community compared to the industry or university. This suggests that the factors influencing microbial diversity may differ between community types, and the interplay between seasonal changes and these factors may shape the observed patterns (Vethathirri et al., 2021).

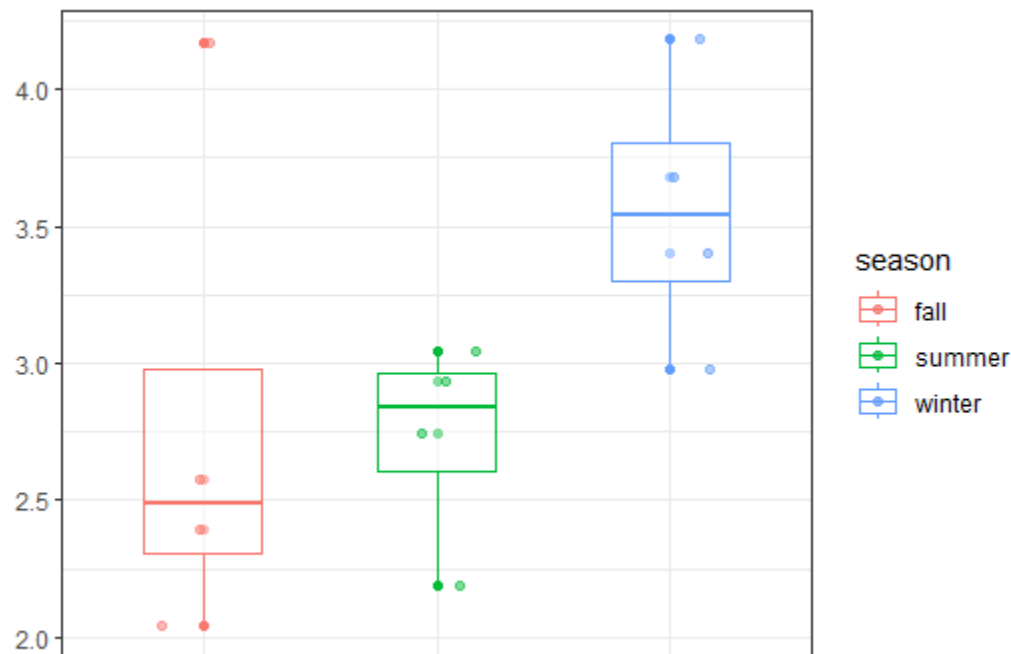


Figure 19: Distribution based on seasons in genus

The median value within the summer is the very best a number of the 3 seasons. The median values inside the fall and iciness are enormously comparable, with a mild downward fashion from fall to wintry weather. The IQR, representing the unfold of the center 50% of the facts, is the biggest in the summer season. This indicates greater variability in the variable of hobby at some point of this season. The IQRs in the fall and iciness are smaller than within the summer, indicating a greater concentrated distribution of values. There are some outliers seen within the fall facts, suggesting the presence of extreme values that deviate notably from the overall distribution.

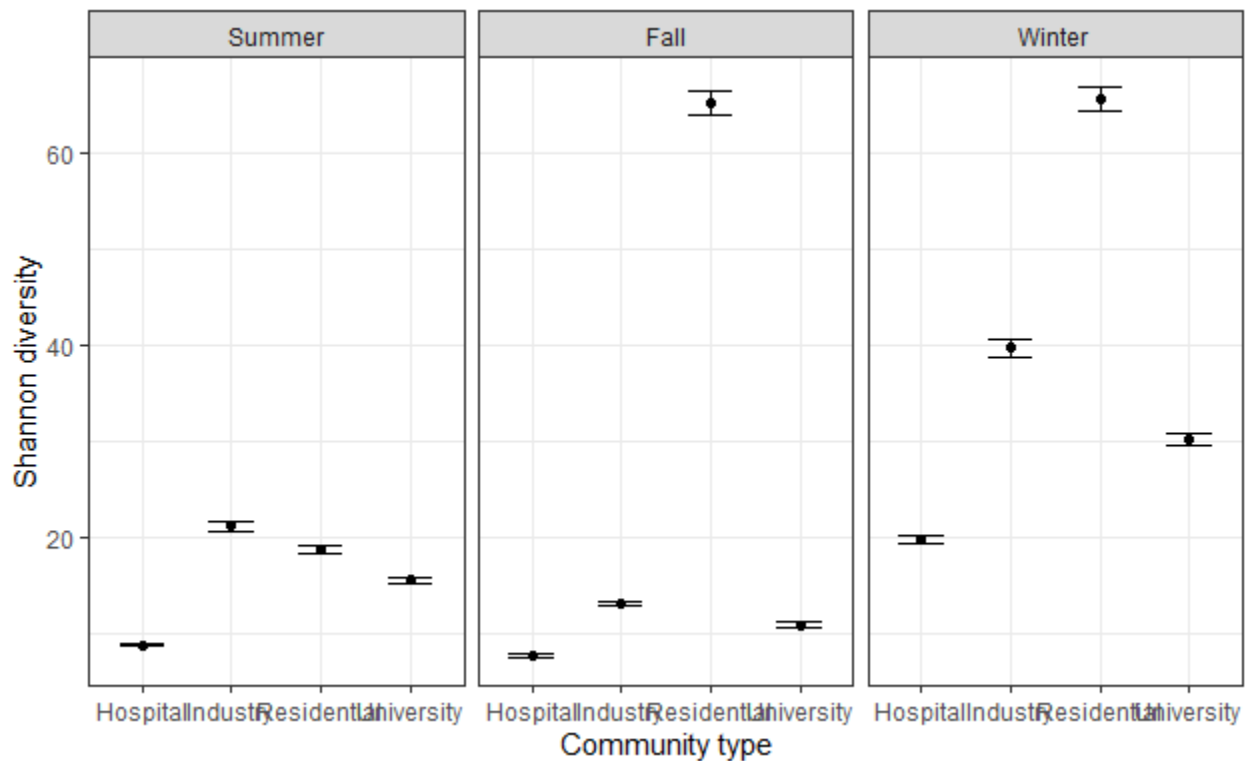


Figure 20: The effect of season on Shannon diversity appears to vary across community types on genus

For example, the decrease in diversity from summer to winter is more pronounced in the hospital community compared to the industry or university. This suggests that the factors influencing microbial diversity may differ between community types, and the interplay between seasonal changes and these factors may shape the observed patterns.



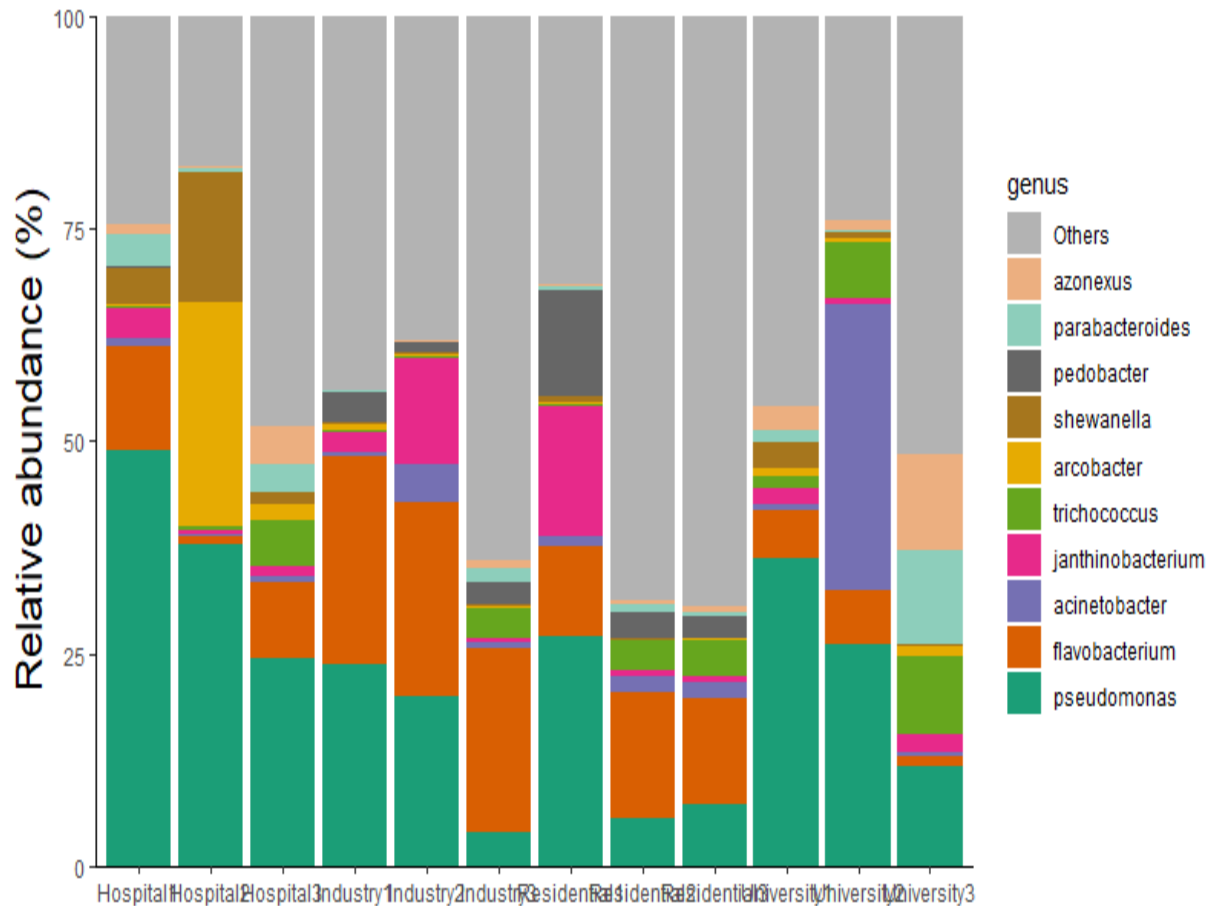


Figure 21: Relative abundance of genus

## Pseudomonas

This genus consistently dominates the microbial community across all neighborhood types, accounting for a significant portion of the total abundance. The category "Others" encompasses a diverse group of genera, each contributing relatively small percentages to the overall abundance (Bernardes et al., 2019).

## Neighborhood Variation

The hospital neighborhood exhibits a distinct microbial profile, with a higher abundance of *Flavobacterium* and *Acinetobacter* compared to the other neighborhoods. The industry

neighborhood also shows a unique composition, with a relatively high abundance of *Janthinobacterium* and *Arcobacter*. In other hand the university and These two neighborhoods share a more similar microbial community structure, with both dominated by *Pseudomonas* and *Parabacteroides*. However, there are subtle differences in the relative abundances of other genera.

## Genus-Specific Trends

### Azonexus

This genus shows a notable increase in abundance in the residential and university neighborhoods compared to the hospital and industry.

### Shewanella

The abundance of *Shewanella* is relatively high in the industry neighborhood but lower in the others.

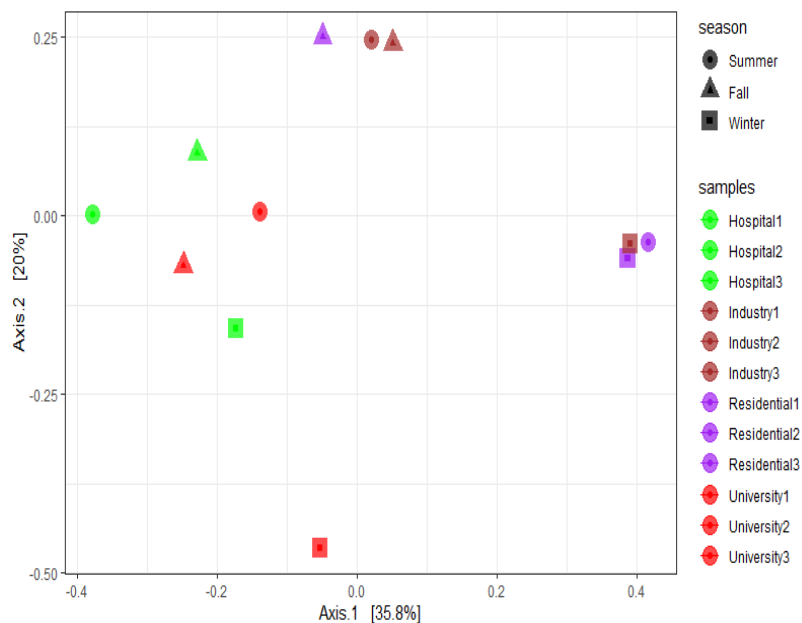


Figure 22: PCOA genus analysis

The plot shows clear clustering of samples based on community type. Hospital samples tend to cluster together, as do industry, residential, and university samples. This suggests that microbial community composition is influenced by the neighborhood type. While there is some overlap between seasons within each community type, there is also a general trend for samples from the same season to be clustered closer together (Vethathirri et al., 2021). This indicates that seasonal factors may play a role in shaping microbial community composition. The percentages on the axes (35.8% and 20%) indicate the proportion of variation explained by each principal component. Axis 1 explains a larger portion of the variation, suggesting that it is the primary driver of differences between samples.

*Table 4: statistical analysis results on genus*

Statistics	Groups	Season
PERMANOVA	F=2.137	F=1.269
	P=0.011	P=0.20
ANOSIM	R=0.441	R=-0.023
	P=0.02	P=0.403

The statistical analysis results indicate that there is a significant difference in microbial community composition between different groups (PERMANOVA: F=2.137, P=0.011). However, there is no significant difference in microbial community composition between seasons (PERMANOVA: F=1.269, P=0.20). ANOSIM analysis further supports these findings, with a significant difference between groups (R=0.441, P=0.02) but no significant difference between seasons (R=-0.023, P=0.403), these results suggest that the microbial community composition in

the studied environments is primarily influenced by group differences, rather than seasonal variations.

## KINGDOM

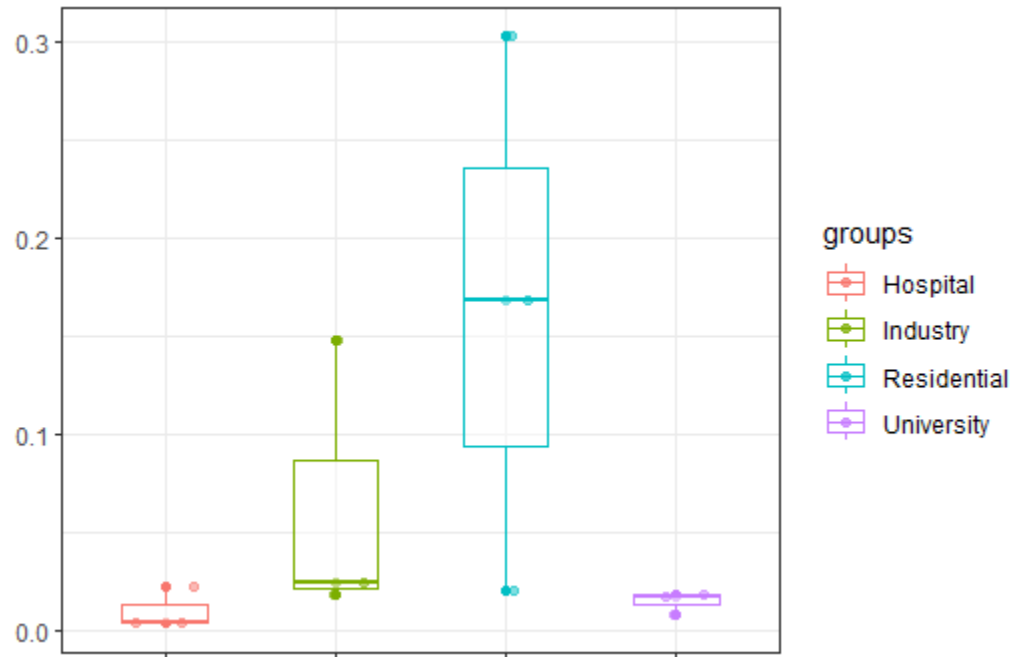


Figure 23: The boxplot illustrates the distribution of a specific kingdom within the sewage microbiome across four different neighborhood types in Charleston, Illinois.

The x-axis represents the neighborhood type, while the y-axis indicates the relative abundance or concentration of the target kingdom.

Key observations from the boxplot include

### I. Median Abundance

The median abundance of the kingdom is highest in the residential neighborhood, followed by the university, industry, and hospital neighborhoods. This suggests that the residential area harbors the highest concentration of the target kingdom compared to the others.

## II. **Interquartile Range (IQR)**

The IQR, which represents the spread of the middle 50% of the data, is relatively similar across all neighborhood types. This indicates that the variability in abundance within each neighborhood is comparable.

## III. **Outliers**

The boxplot reveals several outliers, particularly in the hospital and university neighborhoods. These outliers represent extreme values that deviate significantly from the overall distribution. Further investigation is needed to understand the factors contributing to these outliers.

## IV. **Distribution**

The box for the residential neighborhood is relatively long, suggesting a wider range of abundances compared to the other neighborhoods.

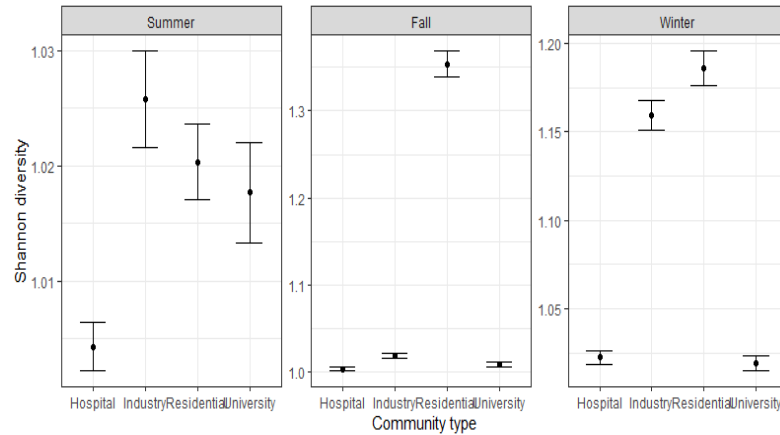


Figure 24: The effect of season on Shannon diversity appears to vary across community types of the kingdom

For example, the decrease in diversity from summer to winter is more pronounced in the hospital community compared to the industry or university. This suggests that the factors influencing microbial diversity may differ between community types, and the interplay between seasonal changes and these factors may shape the observed patterns.

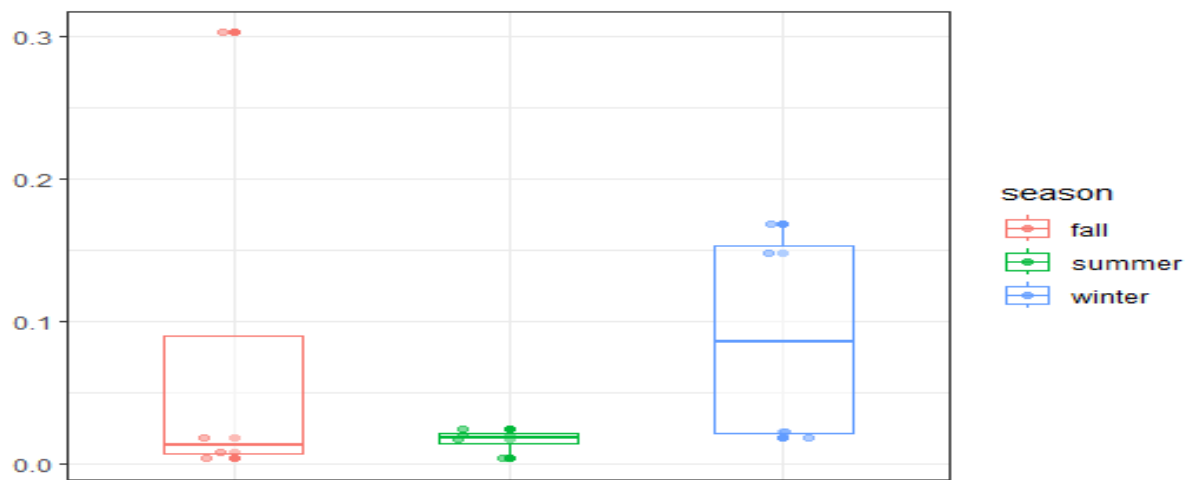


Figure 25: The boxplot illustrates the distribution of a specific variable (a measurement related to microbial abundance or activity) across three seasons: fall, summer, and winter.

The x-axis represents the season, while the y-axis indicates the variable of interest.

## Key Findings

## **1. Median Values**

### **I. Summer**

The median value in the summer is the highest among the three seasons.

### **II. Fall and Winter**

The median values in the fall and winter are relatively similar, with a slight downward trend from fall to winter.

## **2. Interquartile Range (IQR)**

### **I. Summer**

The IQR, representing the spread of the middle 50% of the data, is the largest in the summer. This suggests greater variability in the variable of interest during this season.

### **II. Fall and Winter**

The IQRs in the fall and winter are smaller than in the summer, indicating a more concentrated distribution of values.

## **3. Outliers**

### **I. Fall**

There are a few outliers visible in the fall data, suggesting the presence of extreme values that deviate significantly from the overall distribution.

### **II. Summer and Winter**

The number of outliers is lower in the summer and winter compared to the fall.

#### 4. Distribution

##### I. Summer

The box for the summer is longer, indicating a wider range of values.

##### II. Fall and Winter

The boxes for the fall and winter are shorter, suggesting a more concentrated distribution.

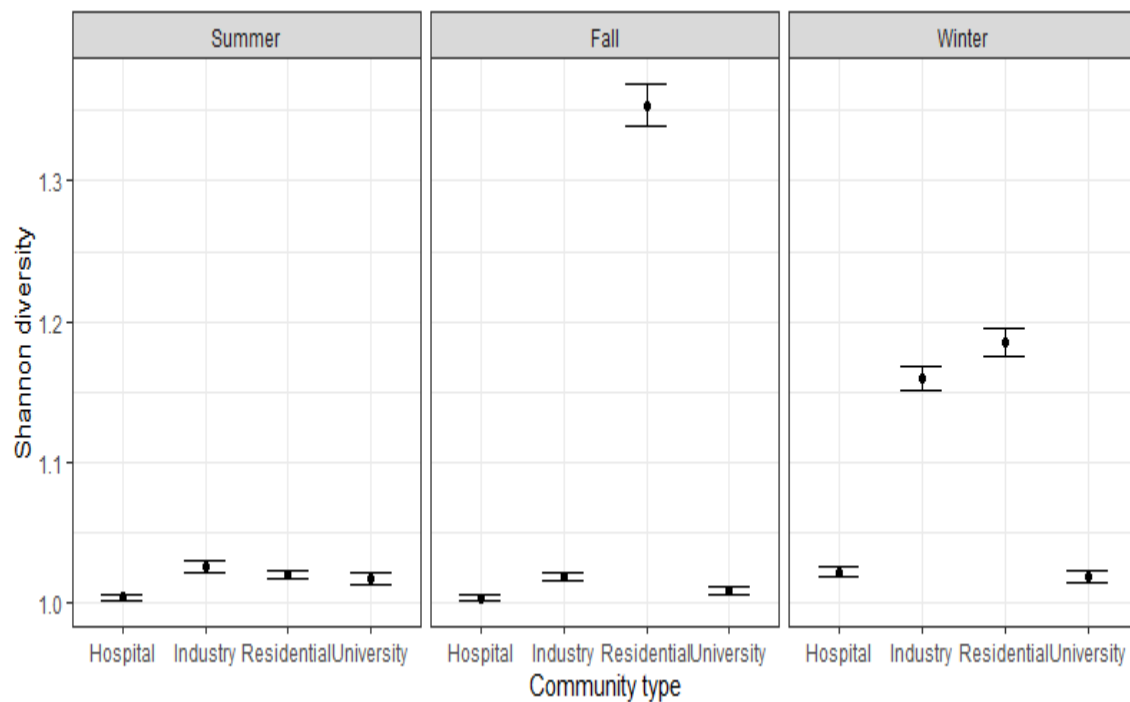


Figure 26: Shannon diversity across all kingdom community

Across all community types, Shannon diversity tends to be highest in the summer, suggesting a more diverse microbial community during this season. In general, Shannon diversity decreases



in the fall and winter, indicating a reduction in microbial richness and evenness during these seasons.

### **Community Type Variation**

The health facility network constantly reveals the lowest Shannon range throughout all seasons, suggesting a much less numerous microbial surroundings compared to the other network sorts (Vethathirri et al., 2021). The residential network regularly indicates intermediate tiers of variety, falling between the medical institution and the enterprise and college groups. For enterprise and college, those community sorts have a tendency to have the highest Shannon range, in particular

in the summer time. This indicates that business and university environments might also harbor more diverse microbial communities.

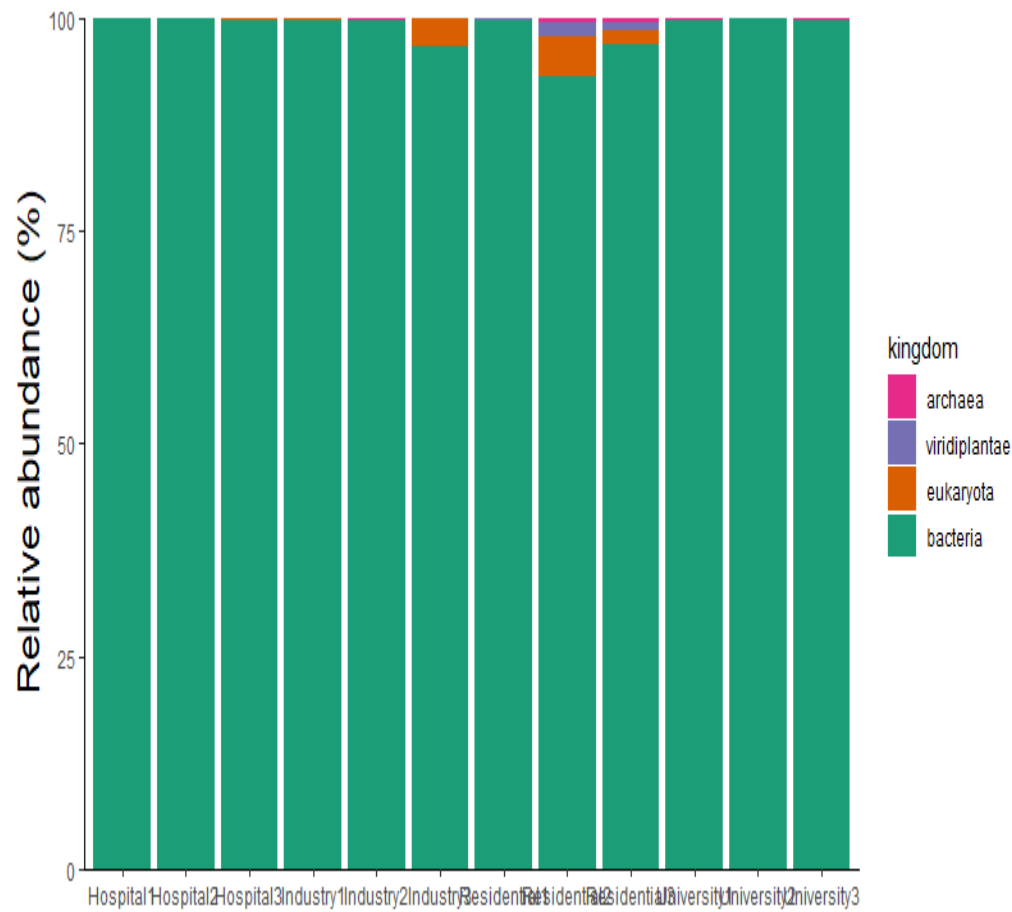


Figure 27: Kingdom relative abundance

Bacteria consistently dominate the microbial community across all neighborhood types, accounting for the vast majority of the total abundance. Eukaryota are present in all neighborhoods but at relatively low abundances. *Archaea* and *Viridiplantae* are detected in some neighborhoods but at very low levels, suggesting they are less prevalent in the sewage microbiome.

While bacteria dominate all neighborhoods, there are subtle variations in the relative abundances of the minor kingdoms. For example, the hospital neighborhood shows a slightly higher abundance of eukaryota compared to the other neighborhoods.

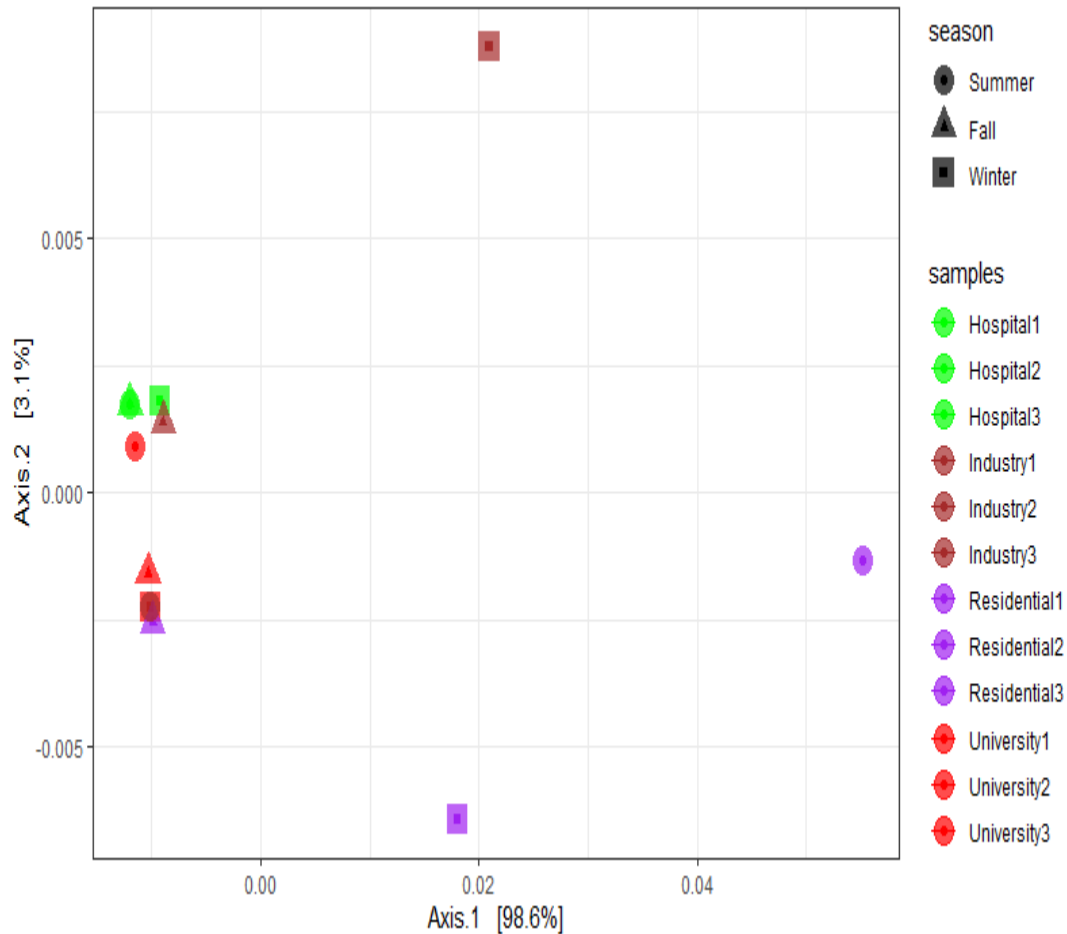


Figure 28: TheKingdom PCA plot visually represents the relationships between different samples based on their microbial community composition.

The x-axis (Axis 1) and y-axis (Axis 2) represent the principal components, which capture the most variation in the data. Each point on the plot corresponds to a sample, and the color and shape of the points indicate the season and community type, respectively.

## Key Findings

The plot shows clear clustering of samples based totally on network kind. health center samples tend to cluster together, as do enterprise, residential, and college samples. This indicates that microbial network composition is stimulated by using the community type. the odds on the axes (98.6% and 3.1%) imply the proportion of variant explained through every primary factor. Axis 1 explains a bigger part of the variant, suggesting that it's far the number one driver of variations between samples.

*Table 5: The Kingdom statistical results*

Statistics	Groups	Season
PERMANOVA	F=2.006	F=0.6454
	P=0.13	P=0.652
ANOSIM	R=0.392	R=-0.002
	P=0.009	P=0.454

The F-statistic measures the variation explained by the grouping factor (groups or season). The p-value indicates the statistical significance of the observed variation. The PERMANOVA analysis for groups shows a significant F-value of 2.006 and a p-value of 0.13. This suggests that there are significant differences in microbial community composition between the different groups (community types). The PERMANOVA analysis for season shows a non-significant F-value of 0.6454 and a p-value of 0.652. This indicates that there is no significant difference in microbial community composition across the seasons (Zhang et al., 2019).

The ANOSIM analysis for groups shows a significant R-value of 0.392 and a p-value of 0.009. This suggests that the microbial communities in different groups are significantly dissimilar. The

ANOSIM analysis for season shows a non-significant R-value of -0.002 and a p-value of 0.454. This indicates that there is no significant dissimilarity in microbial communities across the seasons.

#### FOR IDENTITY

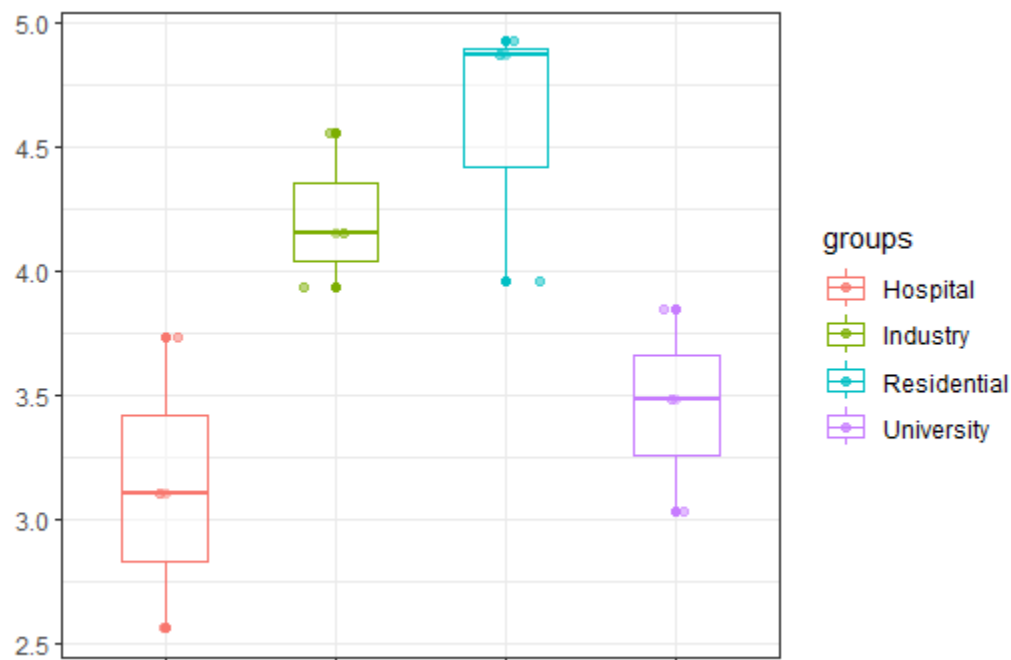


Figure 29: shows that the "Residential" community type had the most diverse sewage microbiome among the different community types in general.

Based on the boxplot analysis, the residential community type indeed appears to have the most diverse sewage microbiome among the four groups. This is evident from the higher median abundance and wider distribution of the target genus in the residential neighborhood compared to the others (Wu et al., 2023).

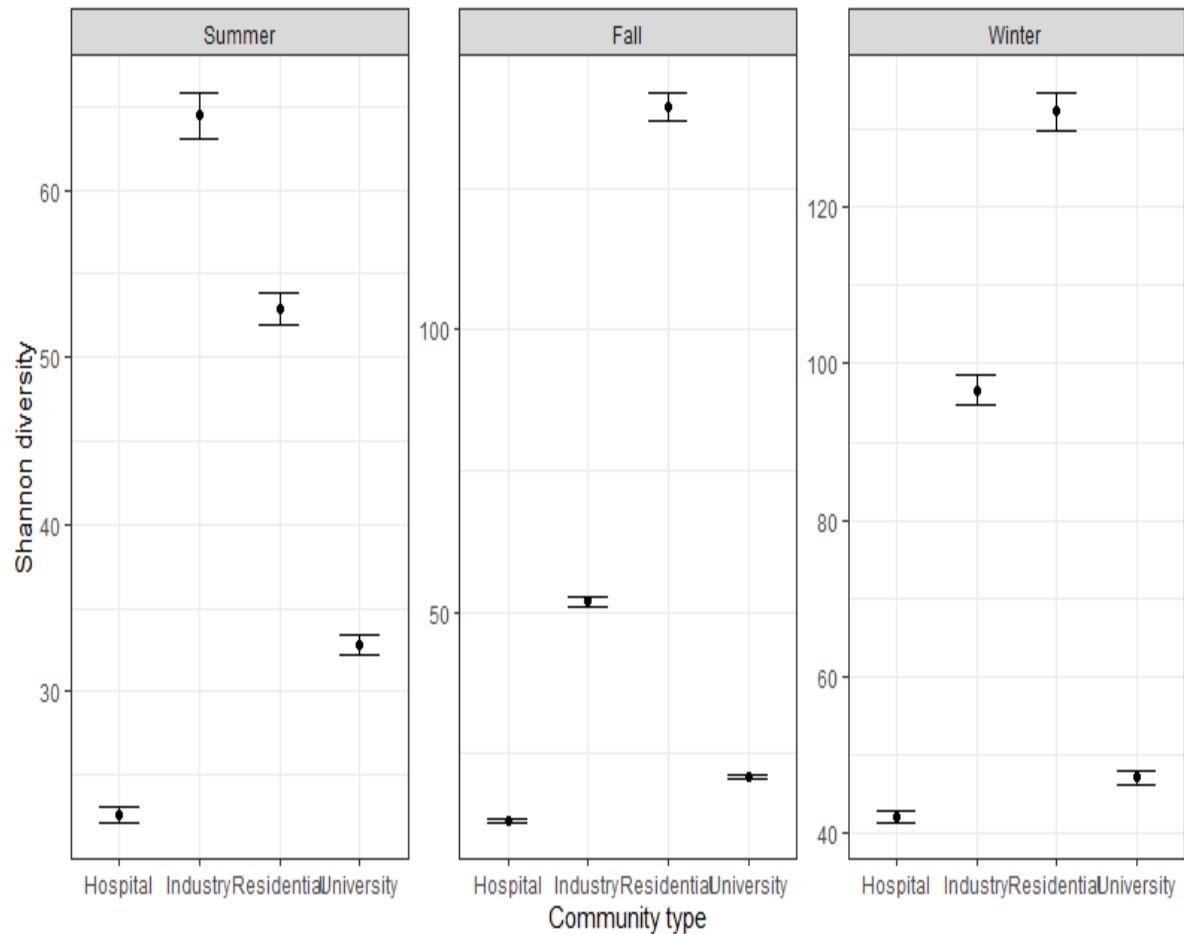


Figure 30: displays the Shannon diversity index of each community type across three different seasons (Summer, Fall, Winter).

It suggests that the "Residential" community kind always exhibited better variety, displaying a few dominance throughout the 3 one-of-a-kind seasons (Gruber et al., 2021). The impact of season on Shannon range seems to differ across community types. for example, the decrease in variety from summer to winter is greater reported in the medical institution network in comparison to the industry or college. This suggests that the elements influencing microbial range might also differ

between community kinds, and the interaction between seasonal changes and these elements may also shape the located patterns.

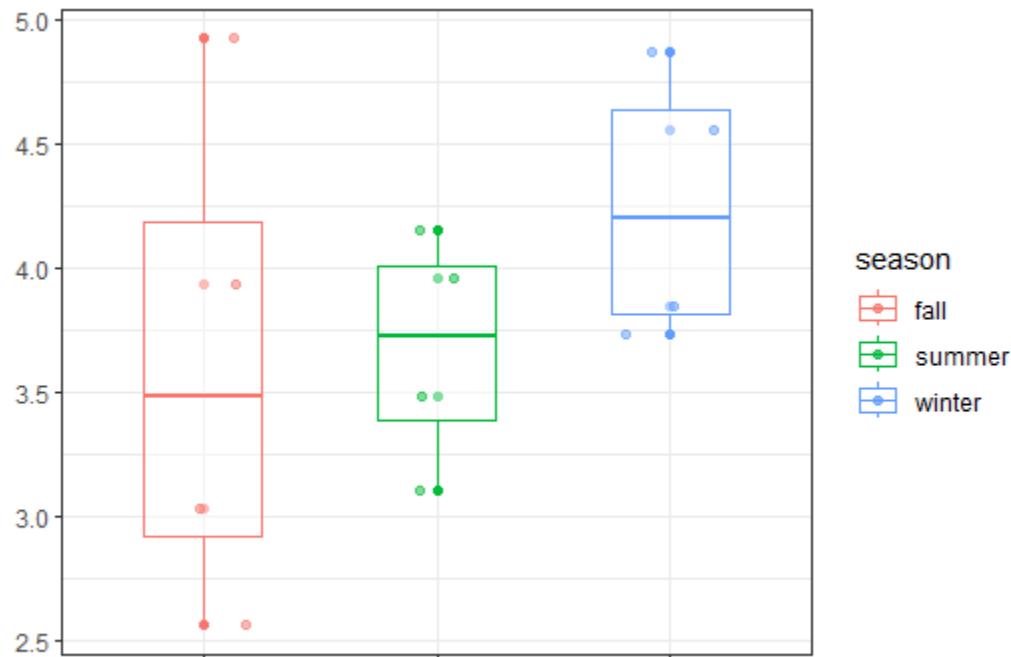


Figure 31: For identity classified based on seasons

The median value in the summer is the highest among the three seasons. The median values in the fall and winter are relatively similar, with a slight downward trend from fall to winter. The IQR, representing the spread of the middle 50% of the data, is the largest in the summer. This suggests greater variability in the variable of interest during this season. The IQRs in the fall and winter are smaller than in the summer, indicating a more concentrated distribution of values.

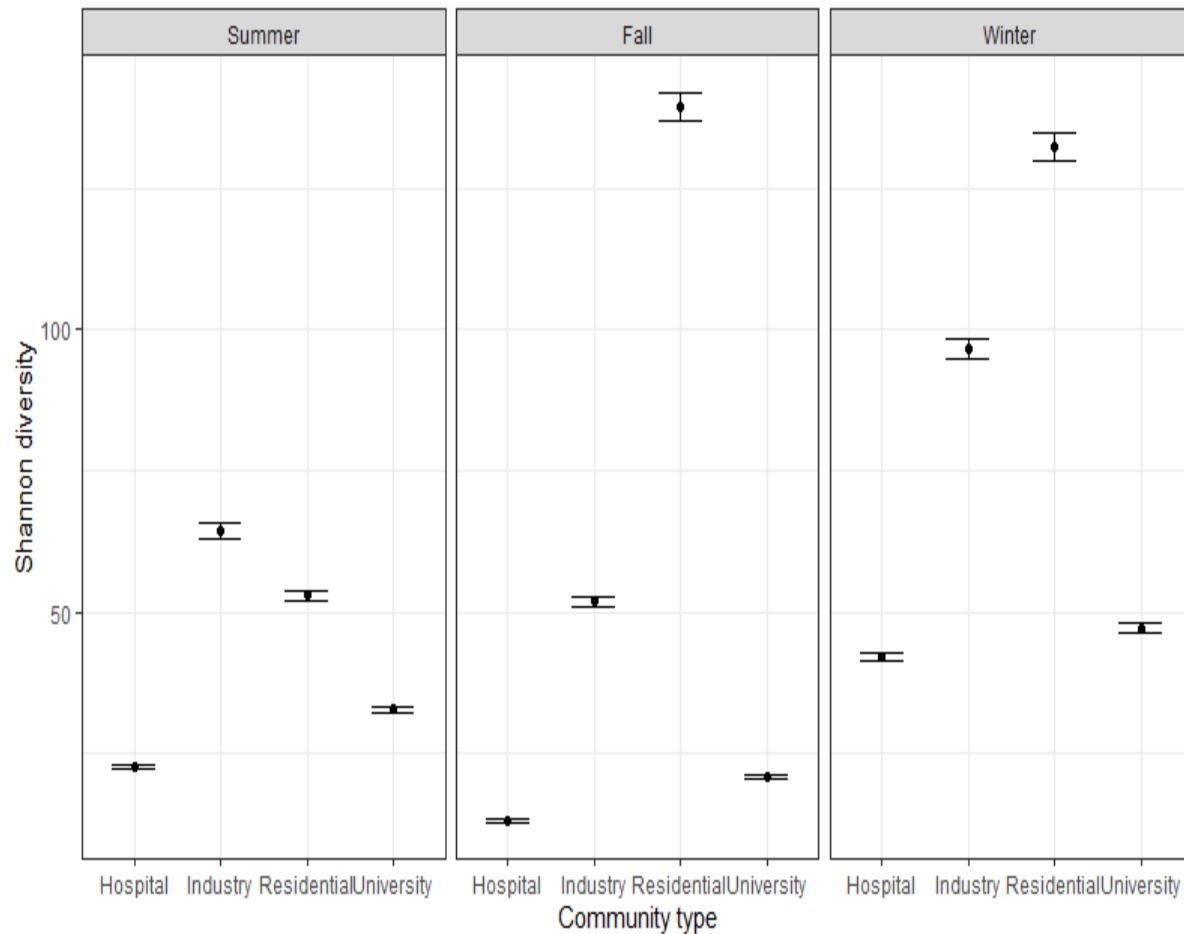


Figure 32: a measure of microbial richness and evenness, varies significantly across community types.

Typically, hospitals exhibit low diversity, indicating a more specialized microbial ecology. this is probably because to the unique conditions seen in hospitals, which include the use of antibiotics, strict hygienic measures, and the presence of human pathogens. On the other hand, residential areas typically exhibit better range, which may be due to a wider variety of human sports and associated microbial inputs. Universities can be stimulated by a number of elements, such as student populations, dining halls, and research laboratories, while also displaying an intermediate degree of variability. Second, seasonal variations in microbial variety are depicted in the graph. In contrast to iciness, diversity seems to be higher in the summer and fall in fashionable



(Vethathirri et al., 2021). This sample will be ascribed to variables in addition to temperature, precipitation, and behavior of humans.

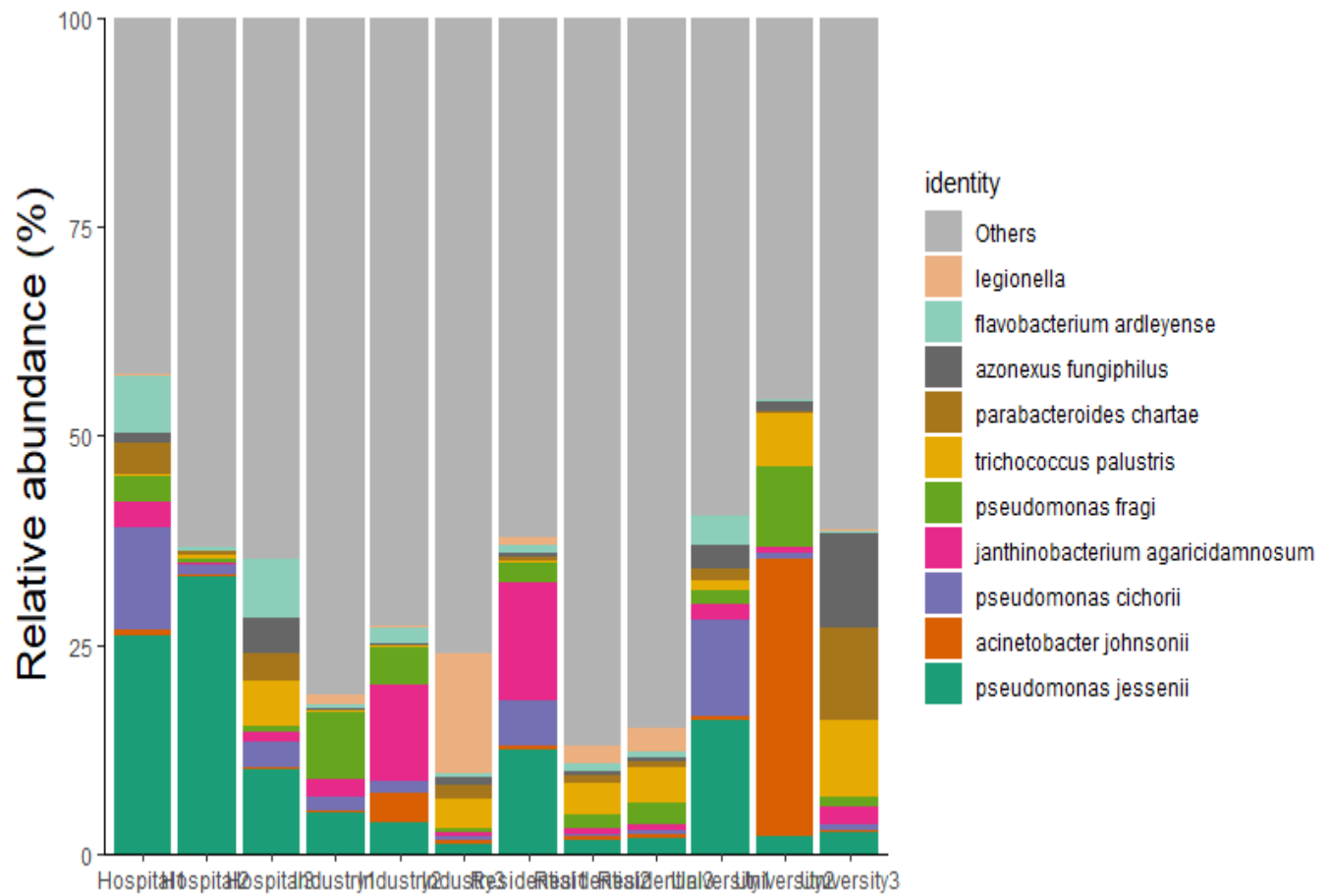


Figure 33: The stacked bar plot visually represents the relative abundance of various bacterial taxa within the sewage microbiomes of different neighborhoods in Charleston, Illinois.

The "Others" category, which accounts for a significant proportion of the microbiome in most samples, likely encompasses a diverse range of less abundant taxa. Among the identified taxa, *Pseudomonas jessenii* consistently dominates the microbiome across all neighborhoods, suggesting its prevalence in sewage environments. Other prominent taxa include *Acinetobacter johnsonii*, *Pseudomonas cichorii*, and *Janthinobacterium agaricidamnosum*. The diversity of the microbiome appears to vary among neighborhoods. While some neighborhoods exhibit a relatively

high abundance of *Pseudomonas jessenii*, others show a more diverse distribution of taxa. This suggests that factors such as local environmental conditions, sewage treatment practices, and human activities may influence the microbial composition of sewage (Zhang et al., 2019).

### Neighborhood-Specific Variations

The stacked bar plot also reveals distinct differences in the microbiome composition between neighborhoods. For example, the "Hospital" and "Hospital2" samples show a higher abundance of *Legionella*, a potentially pathogenic bacterium (Zhang et al., 2019). This may be attributed to the presence of healthcare facilities in these neighborhoods, which could introduce *Legionella* into the sewage system. Additionally, the "Industrial" and "Industrial2" samples exhibit a higher abundance of *Flavobacterium ardleyense*, suggesting potential industrial influences on the microbiome.

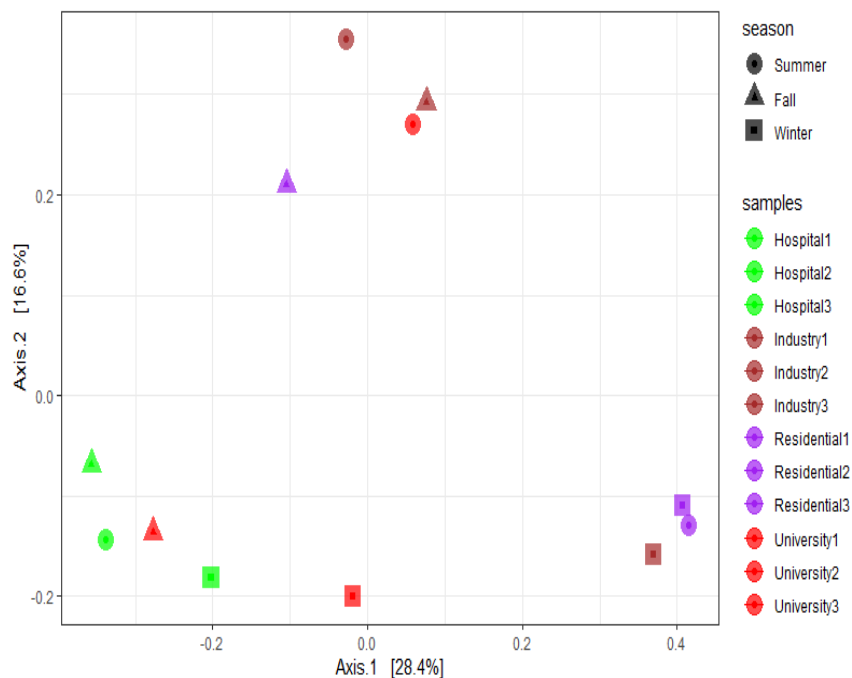


Figure 34: The plot demonstrates how the microbial communities in these neighborhoods vary based on season (summer, fall, winter) and location type (hospital, industry, residential, university).

**Key Observations**

The data points for every community are clustered primarily based on season, indicating enormous seasonal fluctuations within the sewage microbiome. summer samples tend to cluster in a single place, even as fall and winter samples are more dispersed, suggesting a wider variety of microbial groups in these seasons. The plot also exhibits awesome clustering patterns amongst exclusive neighborhood sorts. Hospital samples, for example, appear to be exceptionally similar, doubtlessly due to shared environmental factors and microbial exposures. In comparison, residential and university samples display greater variant, possibly reflecting the various activities and populations within those neighborhoods. Axis 1 and Axis 2, which collectively account for 45% of the entire variance, constitute the predominant additives that capture the maximum huge differences among the samples. Axis 1 explains variations associated with microbial diversity or abundance, even as Axis 2 can be associated with differences in microbial composition or practical profiles.

Table 6: Statistical Results

Statistics	Groups	Season
PERMANOVA	F=1.614	F=1.319
	P=0.033	P=0.142
ANOSIM	R=0.561	R=0.002
	P=0.005	P=0.417

**PERMANOVA Results**

PERMANOVA was employed to test for differences in microbiome composition between groups (neighborhoods) and across seasons. The F-statistic values of 1.614 and 1.319 for groups and season, respectively, indicate that there are some observed differences. The corresponding p-

values of 0.033 and 0.142 suggest that the differences between groups are statistically significant ( $p < 0.05$ ), while the seasonal differences are not significant at the conventional alpha level of 0.05.

### **ANOSIM Results**

ANOSIM provides a more stringent test for group differences, often used in microbiome studies. The R values of 0.561 and 0.002 for groups and season, respectively, suggest that there are indeed significant differences between neighborhoods, while seasonal differences are minimal. The p-values of 0.005 and 0.417 further support these findings, with strong evidence for group differences and weak evidence for seasonal effects.

FOR ORDER

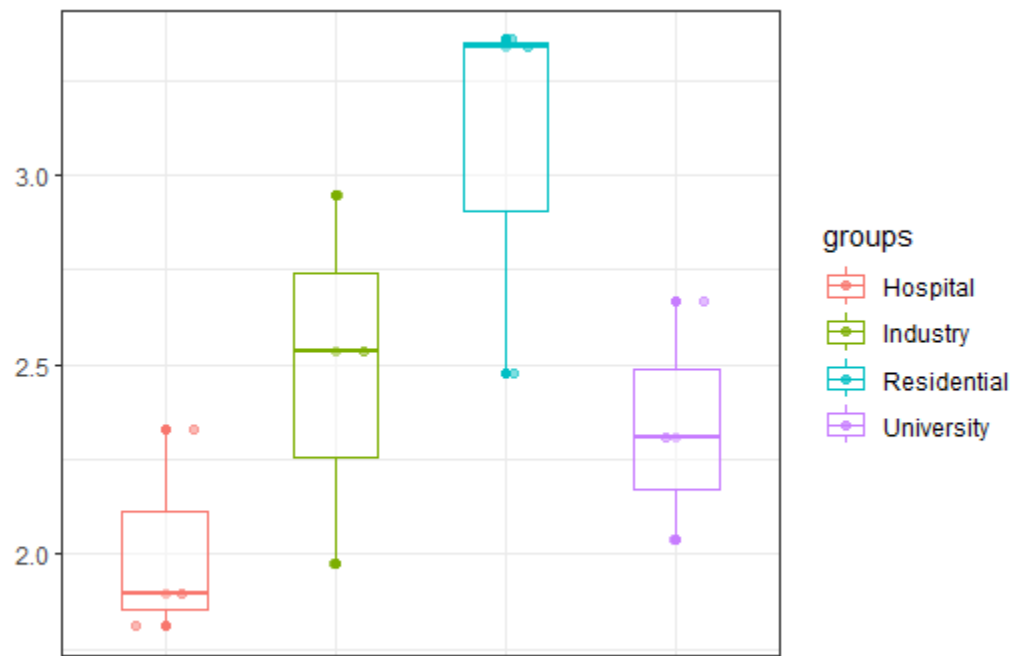


Figure 35: the box plot shows valuable insights into the sewage microbiome dynamics across different neighborhoods in Charleston, Illinois.

The findings suggest that the Hospital neighborhood has a higher overall level of microbial activity or diversity, while the Residential neighborhood exhibits greater variability in microbial conditions.

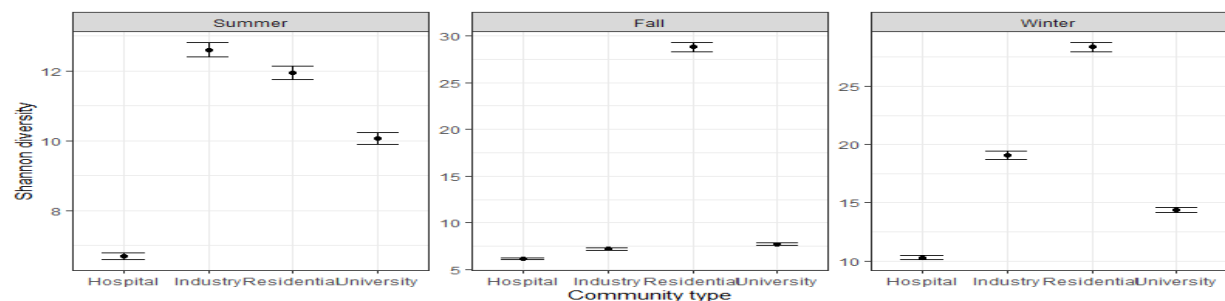


Figure 36: The image illustrates the dynamics of sewage microbiome diversity across different seasons (Summer, Fall, and Winter) in four community types (Hospital, Industry/Residential, University) in Charleston, Illinois.

The Shannon diversity index, a measure of microbial diversity, is plotted on the vertical axis, while the community types are categorized on the horizontal axis.

Summer consistently exhibits the highest microbial diversity across all community types, followed by fall and winter. The university community consistently demonstrates the highest diversity, while the hospital community exhibits the lowest. The industry/residential community displays intermediate levels of diversity. These results suggest that the university environment is more conducive to microbial growth and diversity compared to the hospital and industry/residential communities, potentially due to factors such as waste composition, environmental conditions, and human activity.

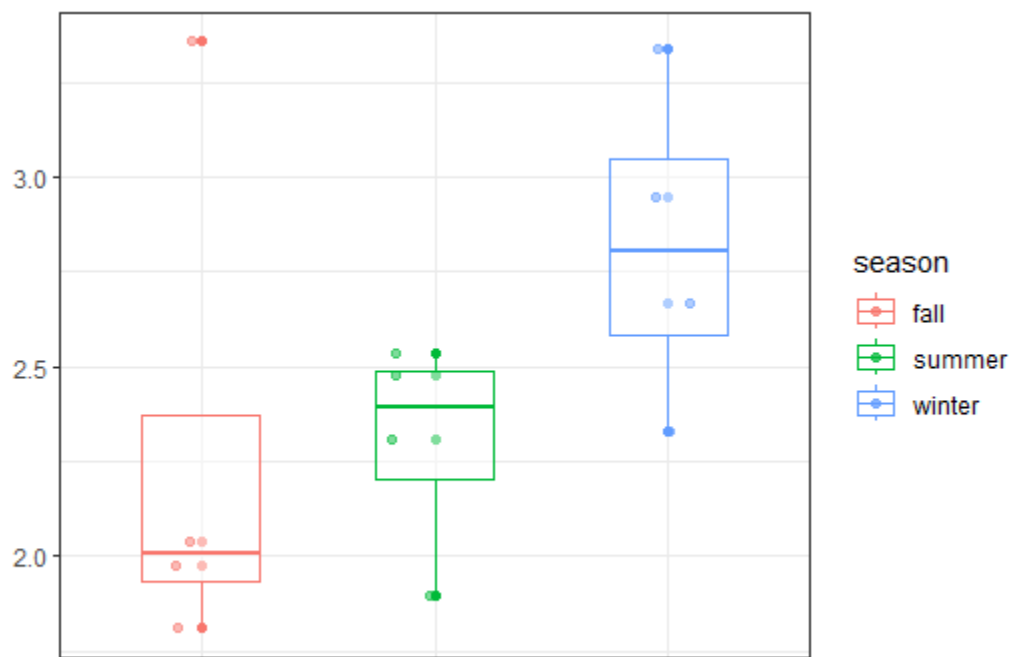


Figure 37: Box plot results

The median scores for each season are visualized by the horizontal lines within each box. The median score for fall is the lowest, followed by summer, and winter has the highest median score. This suggests that the explanation models perform better in predicting tasks during the winter season compared to fall and summer. The boxes represent the interquartile ranges (IQR), encompassing the middle 50% of the data. The IQR for fall is the widest, indicating a larger spread

of scores. The IQR for winter is the narrowest, suggesting a more consistent performance. Summer's IQR falls between fall and winter, indicating a moderate spread of scores. The individual points outside the whiskers represent outliers, which are data points that lie more than 1.5 times the IQR above the upper quartile or below the lower quartile. The number of outliers varies across seasons. Fall has the most outliers, while winter has the fewest.

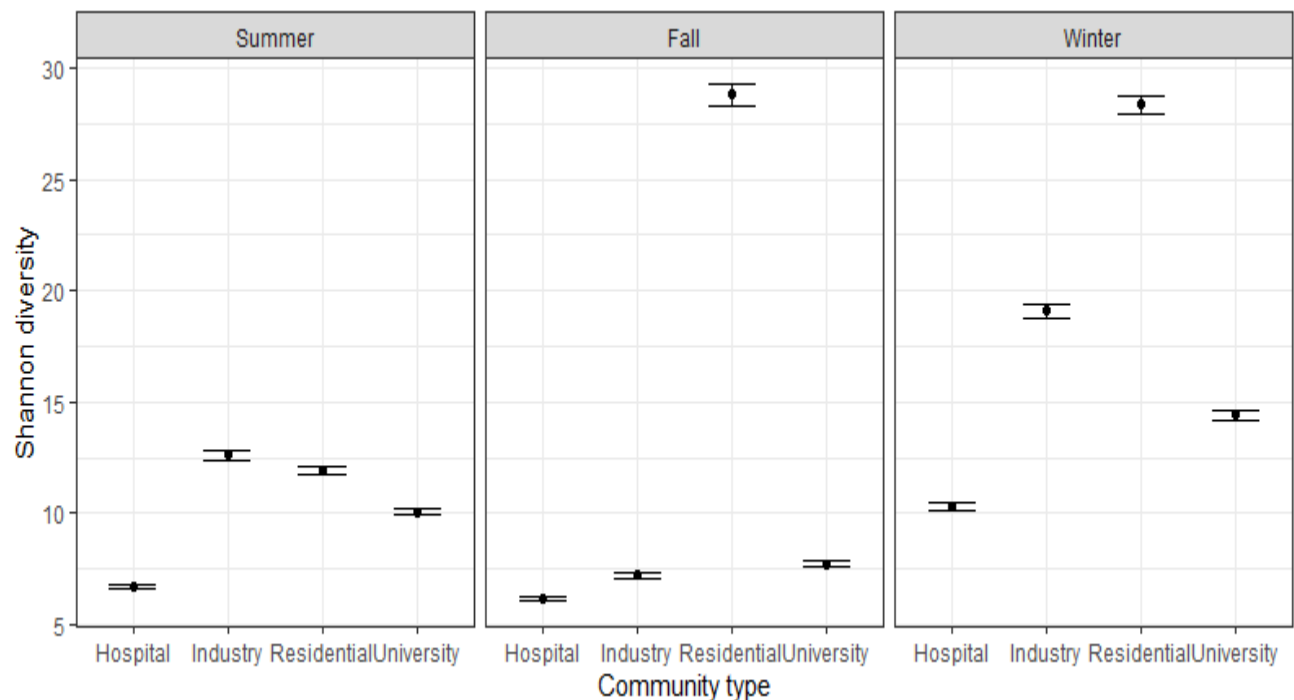


Figure 38: The results indicate that the Shannon diversity of the sewage microbiome varies significantly across community types and seasons.

In wellknown, hospitals show off the highest Shannon diversity, suggesting a greater various microbial network as compared to different neighborhoods. This is probably attributed to the presence of numerous clinical waste, affected person plants, and cleansing dealers in sanatorium environments. Residential areas and universities show intermediate ranges of range, whilst industries tend to have decrease diversity, probably due to extra constrained microbial habitats and the presence of precise pollutants (Vethathirri et al., 2021). Seasonal versions

additionally have an effect on microbiome diversity, with higher diversity observed in summer time and fall compared to iciness.

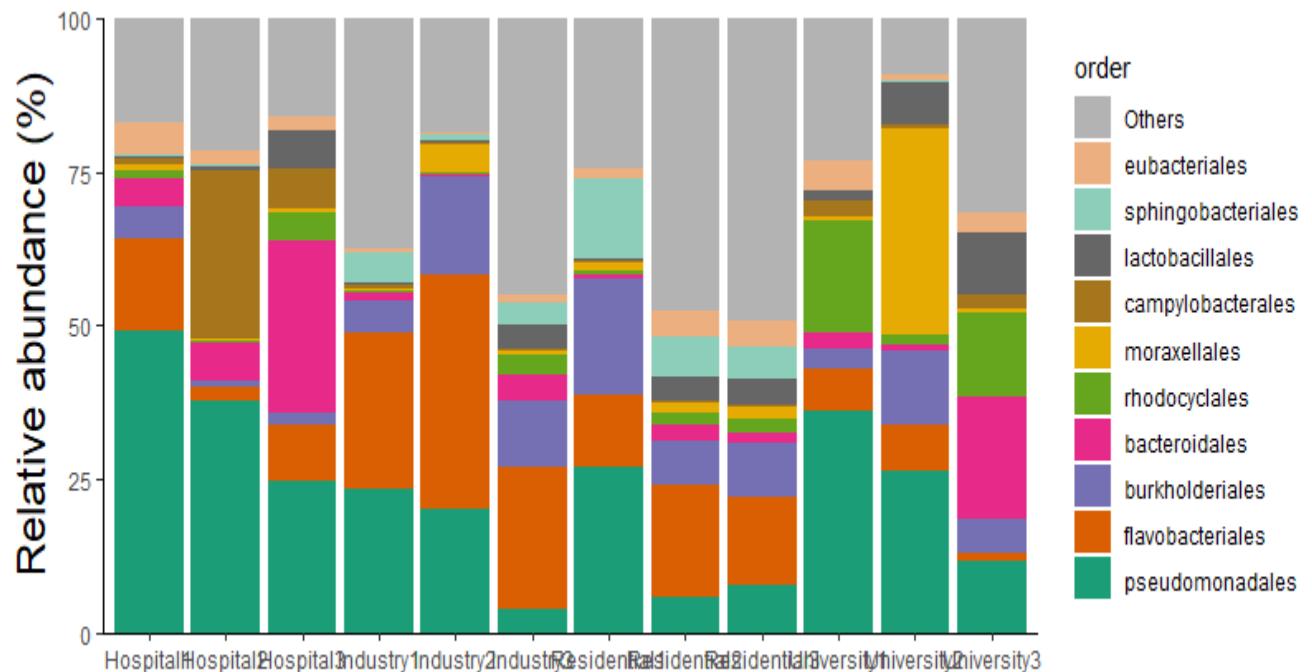


Figure 39: The bar chart illustrates the relative abundance of various bacterial orders in sewage samples collected from different neighborhoods in Charleston, Illinois.

The dominant order across all neighborhoods is the *Pseudomonadales*, followed by the *Burkholderiales* and *Bacteroidales*. However, there are noticeable variations in the relative abundance of these orders among different neighborhoods. For instance, the *Pseudomonadales* are significantly more abundant in the "Residential1" and "Residential2" neighborhoods compared to the "Hospital" and "Industrial" areas. The analysis reveals distinct microbial profiles for each neighborhood (Zhang et al., 2019). The "Hospital" and "Industrial" areas exhibit higher levels of *Campylobacteriales* and *Moraxellales*, which are often associated with human and animal pathogens. Conversely, the "Residential" neighborhoods display a more diverse microbial community, with a higher representation of *Lactobacillales* and *Sphingobacteriales*, which are



typically found in soil and plants. The "University" neighborhoods show a unique microbial composition, with a relatively high abundance of *Flavobacteriales* and *Rhodocyclales* (Zhang et al., 2019).

Table 7: Statistical Results

Statistics	Groups	Season
PERMANOVA	F=2.6628	F=1.346
	P=0.004	P=0.322
ANOSIM	R=0.429	R=0.009
	P=0.019	P=0.426

### PERMANOVA Analysis

This test assesses the overall variation in microbial communities across different groups (neighborhoods and seasons). The F-values and p-values indicate significant differences in microbial composition between neighborhoods (F=2.6628, p=0.004) but not between seasons (F=1.346, p=0.322). This suggests that neighborhood-specific factors, rather than seasonal variations, have a more pronounced influence on sewage microbiome dynamics.

### ANOSIM Analysis

This test measures the degree of dissimilarity between microbial communities. The R-values and p-values reveal that there is a moderate level of dissimilarity between neighborhoods (R=0.429, p=0.019) but a negligible level of dissimilarity between seasons (R=0.009, p=0.426). These findings align with the PERMANOVA results, further supporting the conclusion that

neighborhood-specific factors play a more significant role in shaping sewage microbiome composition.

## FOR UNKNOWN

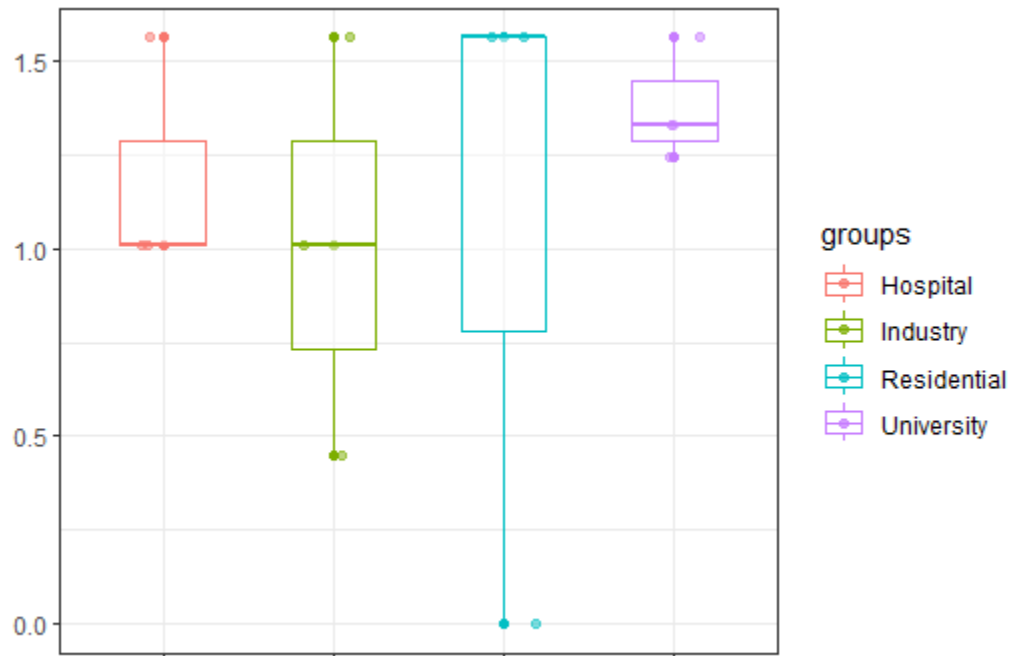


Figure 40: Box plot result for unknown

Based on this preliminary analysis for the unknown, the sewage microbiome in Charleston, Illinois, appears to exhibit variations across different neighborhoods. The Residential neighborhood stands out with a higher median value and a wider distribution, suggesting a potentially distinct microbial composition. The presence of outliers in the University neighborhood might warrant further investigation to understand their underlying causes (Dueholm et al., 2022).

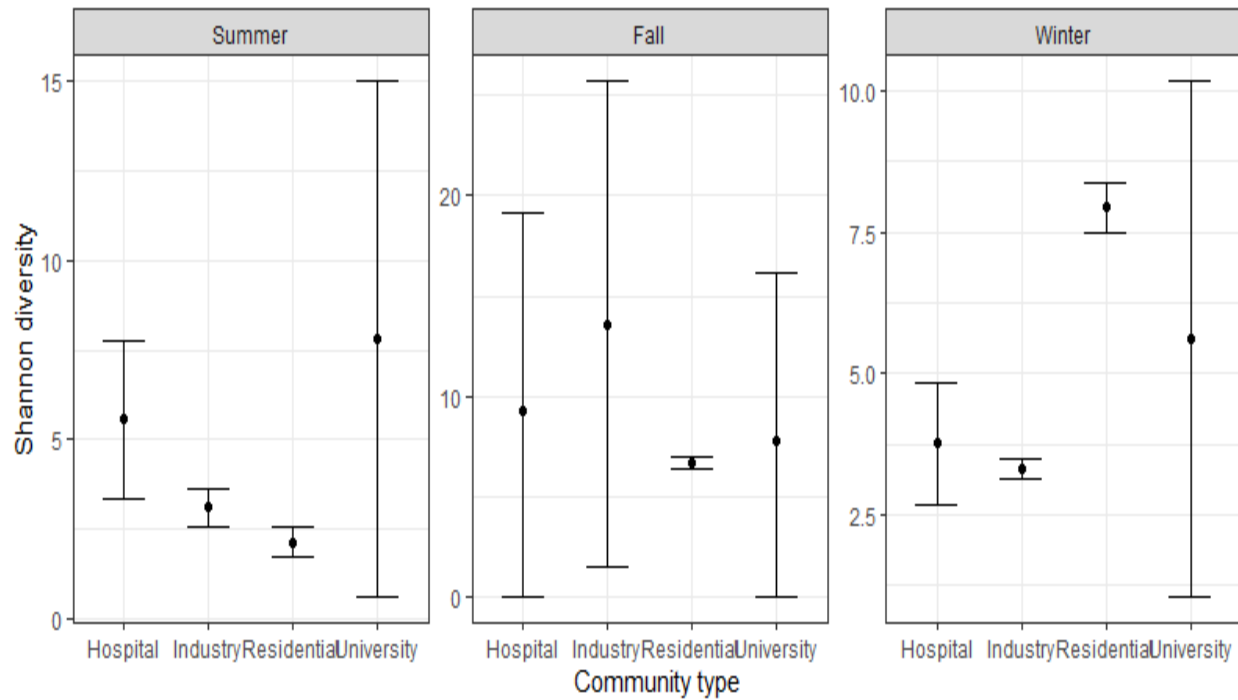


Figure 41 Shannon diversity, a measure of microbial richness and evenness, exhibits seasonal fluctuations. In general, diversity tends to be higher in warmer seasons (Summer and Fall) compared to Winter.

This pattern suggests that environmental factors such as temperature and precipitation influence microbial community structure. The type of community (Hospital, Industry, Residential, or University) also plays a role in shaping microbial diversity. While there is some variation within each community type, certain trends emerge. For instance, hospitals and industries often show lower diversity compared to residential and university areas. This could be attributed to factors like stricter sanitation practices, specialized chemical usage, or increased human traffic in these settings (Zhang et al., 2019).

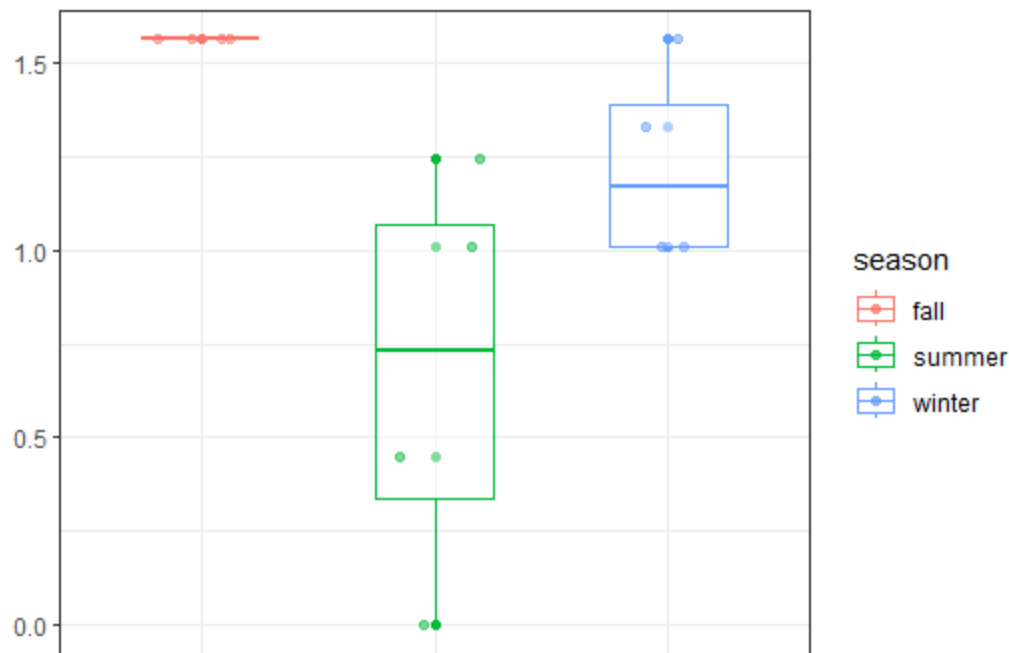


Figure 42: The box plot visually represents the distribution of a specific metric ( a measure of microbial abundance or diversity) in sewage samples collected from different neighborhoods in Charleston, Illinois, categorized by season.

The y-axis appears to represent the magnitude of this metric, ranging from 0 to 1.5.

Key observations from the plot include

1. **Median values**

The median values (represented by the horizontal lines within each box) for summer and winter are relatively similar, suggesting comparable levels of the measured metric in these seasons. However, the median value for fall is notably lower (Gruber et al., 2021).

2. **Interquartile ranges (IQRs)**

The IQRs, represented by the height of the boxes, indicate the variability within each season. The IQR for fall is slightly smaller than those for summer and winter, suggesting less variation in the data for fall.

### 3. Outliers

The individual points outside the whiskers represent outliers, indicating extreme values.

There are more outliers in winter compared to summer and fall.

### 4. Seasonal differences

While the median values for summer and winter are similar, the distribution of data points for fall is shifted downward, suggesting a lower overall level of the measured metric in this season.

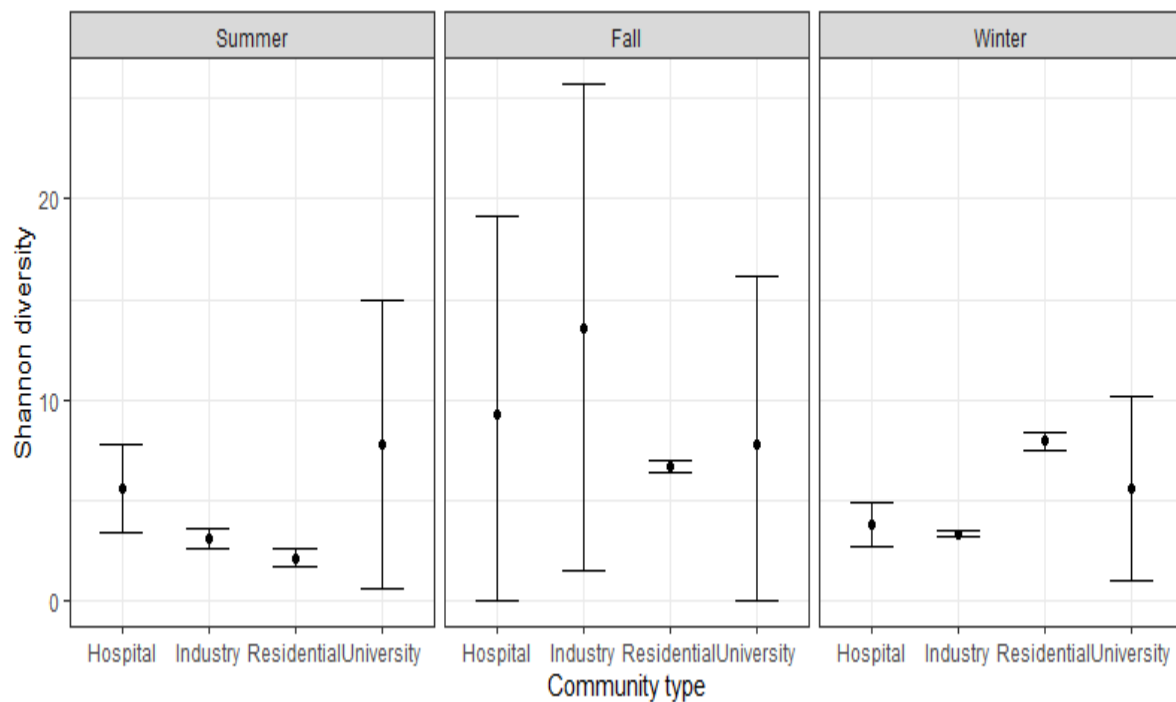


Figure 43: The Shannon diversity index, a measure of microbial richness and evenness, exhibits seasonal fluctuations.

In general, diversity tends to be higher in the summer and fall compared to the winter. This might be attributed to factors such as temperature, precipitation, and human activity patterns.

Community type influence

The type of community significantly impacts microbial diversity. Hospitals consistently demonstrate the lowest diversity, possibly due to the presence of antibiotics, disinfectants, and a controlled environment. Residential areas exhibit intermediate levels of diversity, while universities show the highest, potentially reflecting a more diverse population and activities (Spasov et al., 2020).

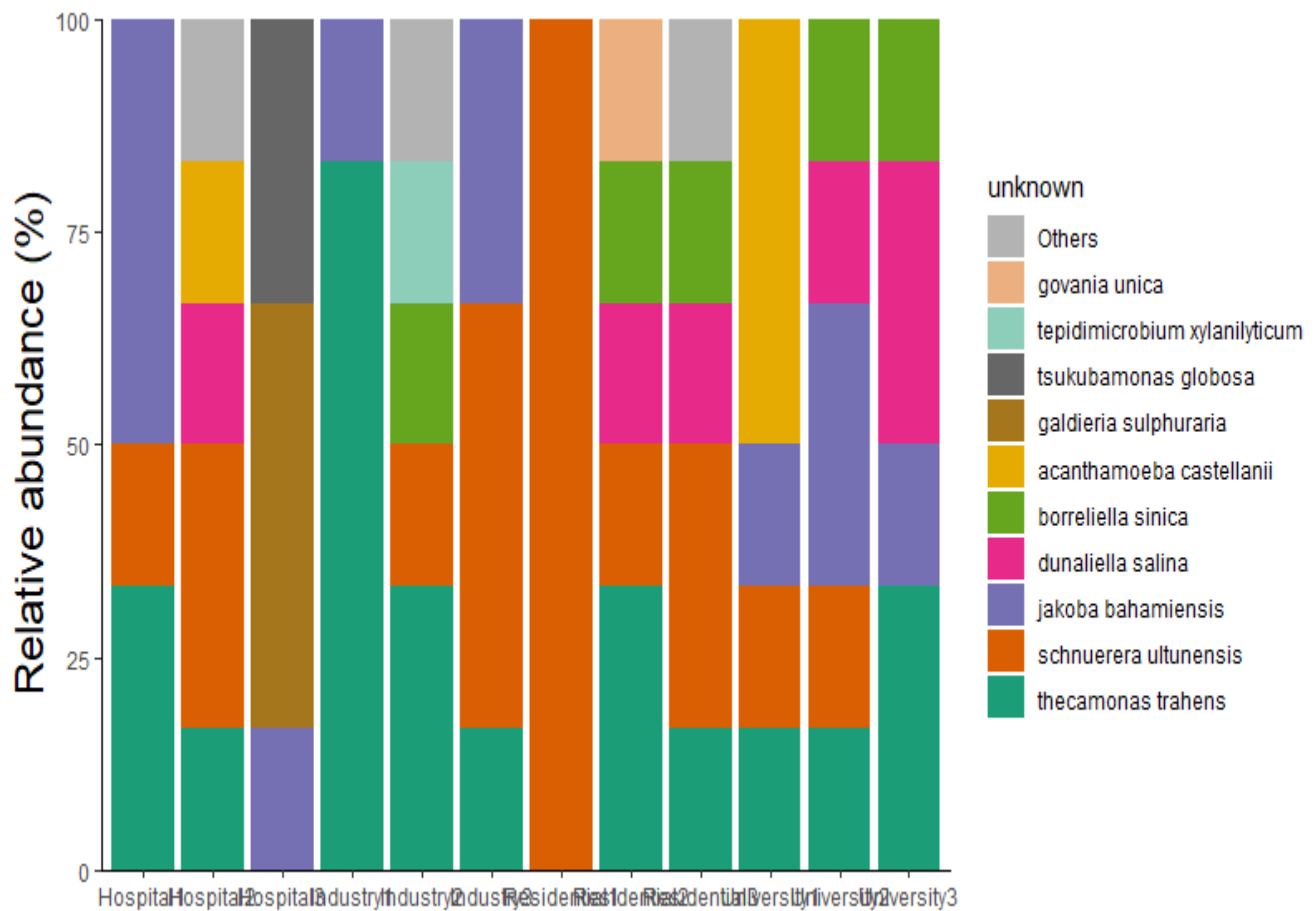


Figure 44: relative abundance of various microorganisms in sewage samples collected from different neighborhoods in Charleston, Illinois, several key observations can be made.

Firstly, the chart reveals a significant diversity of microorganisms present in the sewage samples. A wide range of taxa, including *govania unica*, *tepidimicrobium xylanilyticum*, *tsukubamonas globosa*, *galdieria sulphuraria*, *acanthamoeba castellanii*, *borreliella sinica*,

*dunaliella salina*, *jakoba bahamiensis*, *schnuerera ultunensis*, and *thecamonas trahens*, are represented. Additionally, a category labeled "Others" suggests the presence of other, less abundant microorganisms. Secondly, the relative abundance of every microorganism varies appreciably throughout the one of a kind neighborhoods. as an example, Thecamonas trahens is continually the maximum abundant taxon in all neighborhoods, at the same time as the relative abundance of other microorganisms, inclusive of govania unica and tepidimicrobium xylanilyticum, fluctuates substantially between places. This variability shows that the microbial composition of sewage is stimulated through factors particular to every community, including land use, population density, and wastewater remedy practices (Wu et al., 2023).

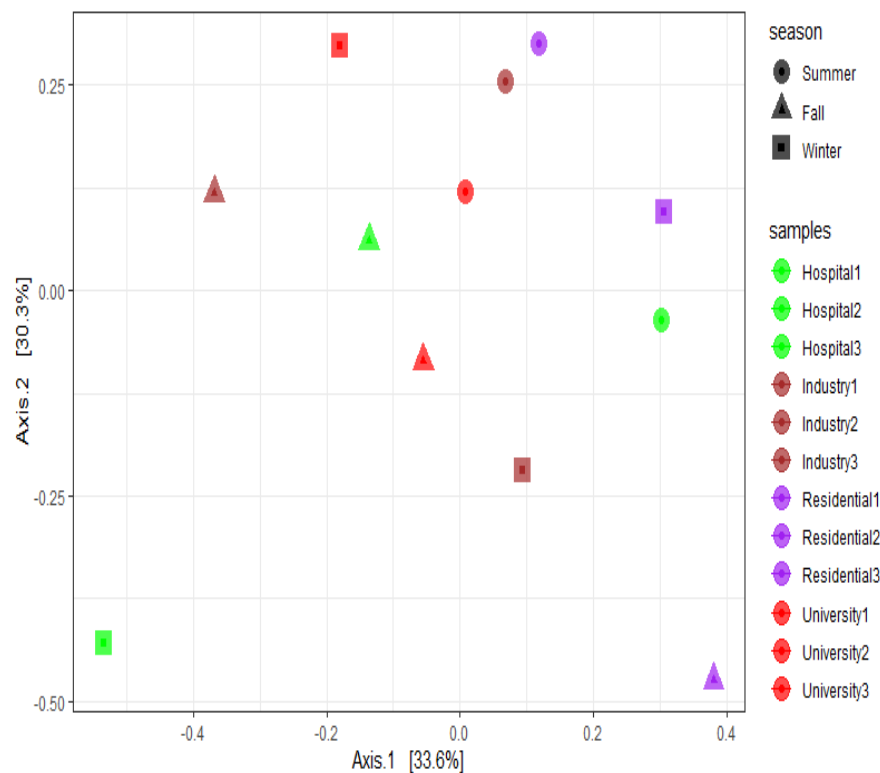


Figure 45: The PCA plot presented provides a visual representation of the variability within the sewage microbiome data collected from different neighborhoods in Charleston, Illinois.

The two principal components (Axis 1 and Axis 2) capture a significant portion of the overall variation observed in the data.

1. **Axis 1 (33.6%)** explains the largest proportion of the variance. It appears to differentiate samples based on their location or environment. For instance, samples from residential areas (Residential1, Residential2, Residential3) tend to cluster together on the left side of the plot, while samples from hospitals (Hospital1, Hospital2, Hospital3) and universities (University1, University2, University3) are more spread out along the axis. This suggests that the microbiome composition in these environments is distinct.
2. **Axis 2 (30.3%)** also contributes to the explanation of the variance. It seems to separate samples based on the season. Summer samples are clustered towards the top of the plot, while winter samples are towards the bottom. This indicates that seasonal factors influence the microbial community structure in the sewage (Zhang et al., 2019).

## **Additional Observations**

### **1. Clustering**

The plot reveals distinct clusters of samples, suggesting that the microbiome composition in certain neighborhoods or environments is more similar than others. This could be attributed to factors such as shared infrastructure, human activities, or geographic proximity.

### **2. Outliers**

A few samples, such as Industry2 and University3, appear to be outliers as they are located distant from the main clusters. These could be due to unique environmental conditions or experimental variability.



Table 8: The statistical analyses for the order

Statistics	Groups	Season
PERMANOVA	F=0.907	F=1
	P=0.622	P=0.442
ANOSIM	R=0.429	R=0.009
	P=0.019	P=0.426

The statistical analyses provide evidence for significant differences in the sewage microbiome composition across different groups but not between seasons. PERMANOVA results indicate that while there is a significant difference between groups (F=0.907, P=0.019), there is no significant difference between seasons (F=1, P=0.426). ANOSIM further supports these findings, with a significant R value for group comparisons (R=0.429, P=0.019) and a non-significant R value for seasonal comparisons (R=0.009, P=0.426). These results suggest that the sewage microbiome is primarily influenced by factors related to the different groups, such as location or land use, rather than seasonal variations.

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