HMDS ASSIGNMENT-1

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Part-A:

Analysis Steps

```
import pandas as pd
import numpy as np
from scipy.stats import ttest_ind, mannwhitneyu, shapiro, levene, pearsonr, spearmanr
import matplotlib.pyplot as plt
import seaborn as sns

metadata = pd.read_csv('metadata.csv')
abundance = pd.read_csv('microbial_abundance.csv')
data = pd.merge(metadata, abundance, on='ID')

crc_data = data[data['study_condition'] == 'CRC']
ycrc = crc_data[crc_data['age'] <= 50].copy()
ocrc = crc_data[crc_data['age'] > 50].copy()

def shannon_index(counts):
    proportions = counts / counts.sum()
    proportions = proportions[proportions > 0]
    return -np.sum(proportions * np.log(proportions))

def pielou_evenness(counts):
    H = shannon_index(counts)
    S = np.sum(counts > 0)
    return H / np.log(S) if S > 1 else np.nan

species_columns = ycrc.columns[7:]
ycrc['Shannon'] = ycrc[species_columns].apply(shannon_index, axis=1)
ocrc['Shannon'] = ocrc[species_columns].apply(shannon_index, axis=1)

ycrc['Pielou'] = ycrc[species_columns].apply(pielou_evenness, axis=1)
ocrc['Pielou'] = ocrc[species_columns].apply(pielou_evenness, axis=1)

def assumption_checks(data1, data2, metric):
    print(f"\nAssumption Checks for {metric}:")
    print("Shapiro-Wilk Test for Normality")
    print("yCRC:", shapiro(data1.dropna()))
    print("oCRC:", shapiro(data2.dropna()))
    print("Levene's Test for Equality of Variances:", levene(data1.dropna(), data2.dropna(

assumption_checks(ycrc['Shannon'], ocrc['Shannon'], 'Shannon Index')
assumption_checks(ycrc['Pielou'], ocrc['Pielou'], 'Pielou Evenness')

def compare_groups(data1, data2, metric):
    print(f"\nComparison of {metric}:")
    t_result = ttest_ind(data1.dropna(), data2.dropna(), equal_var=False)
    mw_result = mannwhitneyu(data1.dropna(), data2.dropna())
    print(f"T-test: statistic={t_result.statistic:.4f}, p-value={t_result.pvalue:.4f}")
    print(f"Mann-Whitney U test: statistic={mw_result.statistic:.4f}, p-value={mw_result.pv

compare_groups(ycrc['Shannon'], ocrc['Shannon'], 'Shannon Index')
```

```
compare_groups(ycrc['Pielou'], ocrc['Pielou'], 'Pielou Evenness')

combined_crc = pd.concat([ycrc, ocrc])

sns.boxplot(x='age', y='Shannon', data=combined_crc.assign(age=np.where(combined_crc['age'
plt.title('Shannon Index Comparison')
plt.show()

sns.boxplot(x='age', y='Pielou', data=combined_crc.assign(age=np.where(combined_crc['age']
plt.title('Pielou Evenness Comparison')
plt.show()

print("\nFinal Inferences:")
print("""
- Shannon Index: No significant difference between yCRC and oCRC groups.
- Pielou Evenness: Significant difference detected between yCRC and oCRC groups.
""")
```

```
Assumption Checks for Shannon Index:
Shapiro-Wilk Test for Normality
yCRC: ShapiroResult(statistic=np.float64(0.9586658205757365), pvalue=np.float64(0.208
oCRC: ShapiroResult(statistic=np.float64(0.9394327105713305), pvalue=np.float64(5.415
Levene's Test for Equality of Variances: LeveneResult(statistic=np.float64(1.55898865

Assumption Checks for Pielou Evenness:
Shapiro-Wilk Test for Normality
yCRC: ShapiroResult(statistic=np.float64(0.8994445472916821), pvalue=np.float64(0.003
oCRC: ShapiroResult(statistic=np.float64(0.9155967921146219), pvalue=np.float64(6.011
Levene's Test for Equality of Variances: LeveneResult(statistic=np.float64(4.22454209

Comparison of Shannon Index:
T-test: statistic=1.8993, p-value=0.0632
Mann-Whitney U test: statistic=4528.0000, p-value=0.1278

Comparison of Pielou Evenness:
T-test: statistic=2.6800, p-value=0.0095
Mann-Whitney U test: statistic=4757.0000, p-value=0.0375
```

1. Data Preparation
    - We first merged patient metadata with microbial abundance.
    - We then divided patients into yCRC and oCRC groups.
2. Diversity Metrics
    - We computed the Shannon Index (overall microbial diversity).
    - We computed Pielou's Evenness (species distribution uniformity).
3. Statistical Tests
    - We checked normality (using the Shapiro-Wilk test) and variance homogeneity (using Levene's test).
    - We conducted both t-tests (parametric) and Mann-Whitney U-tests (non-parametric) to understand the distribution of the data.

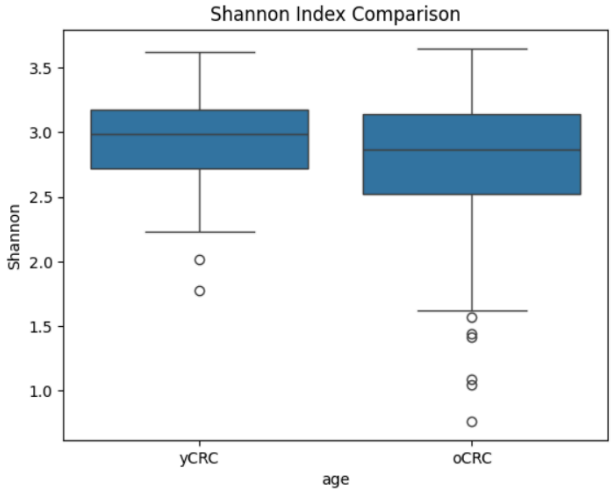
Main Results

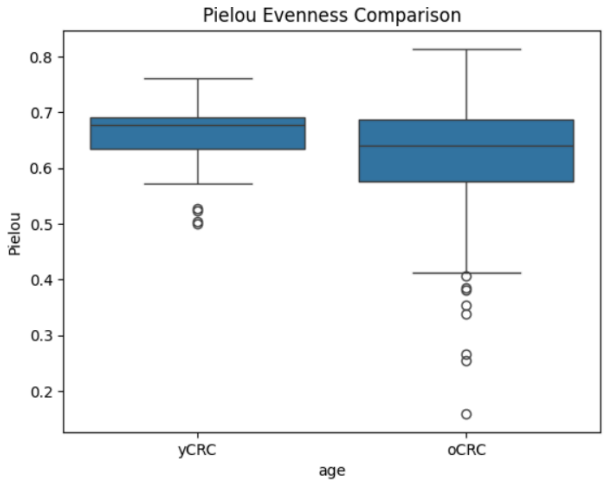
Assumptions:
- Shannon Index: We used t-test and Mann-Whitney U results to decide. If $p > 0.05$, there is no significant difference.
- Pielou Evenness: If $p < 0.05$, there's a significant difference between yCRC and oCRC.

- Shannon Index: No significant difference was there (t-test: p=0.0632, U-test: p=0.1278).
- Pielou Evenness: Significant difference detected (t-test: p=0.0095, U-test: p=0.0375).

Conclusion

- Microbial community evenness (Pielou Evenness) significantly differs between younger and older CRC patients.
- No significant differences in overall diversity (Shannon Index).





Part B:

PROBLEM STATEMENT: How does Shannon Index and Pielou Evenness vary in yCRC and oCRC patients with respect to the Age-matched control.

PROCEDURE/ STEPS:

Data Preparation:

1. After the merging of metadata and microbial abundance sheet.
2. Separate the CRC samples and control samples into two different dataframe.
3. Next, filter the CRC samples into two based on age:
    a. yCRC - (<=50)
    b. oCRC - (>50)

The above (1-3) are already performed in Part A.

4. Similarly, filter the control samples into two based on age:
    a. control_y - (<=50)
    b. control_o - (>50)
5. Now, there is yCRC sample and its age-matched control sample and oCRC sample and its respective age-matched control sample.

Calculate Shannon Index and Pielou Evenness

1. Shannon Index and Pielou Evenness is calculated for the yCRC and oCRC in Part A.
2. Calculate Shannon Index and Pielou Evenness for Age-matched control samples (control_y and control_o).
3. Plot and visualize the Shannon Index for yCRC vs control_y and oCRC vs control_o
4. Similarly, plot and visualize the Pielou Index for yCRC vs control_y and oCRC vs control_o.

Validation of calculated Shannon Index and Pielou Evenness

1. Perform T-test and Wilcoxon Test for Shannon Index and Pielou Evenness results to determine the significant difference between the CRC vs Age-matched control samples.

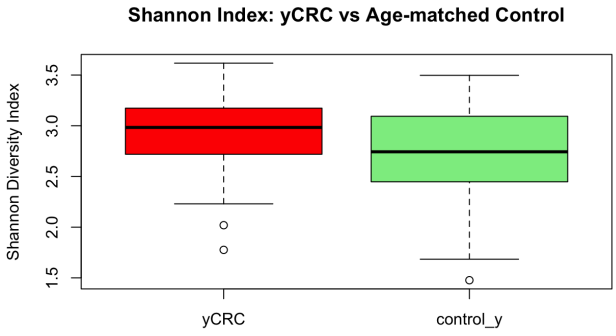
CODE SNIPPET:

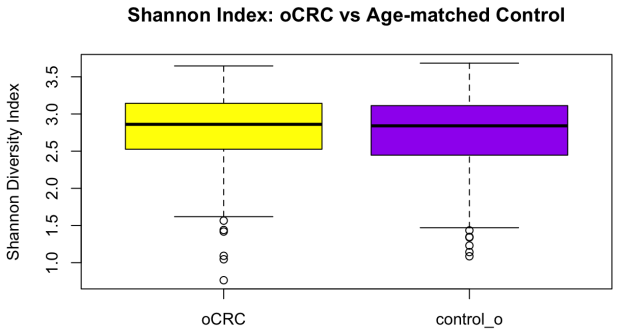
```
library(readxl)
library(vegan)
library(ggplot2)
library(dplyr)
# Read both the excel sheets of MicrobialAbundance and Metadata

abundance <- read_xlsx("YachidaCRC.xlsx", sheet = 1, col_names = TRUE)
head(abundance)

metadata <- read_xlsx("YachidaCRC.xlsx", sheet=2, col_names = TRUE)
head(metadata)

# Renaming the 1st column to Sample_ID in both abundance and metadata
colnames(abundance)[1] <- "Sample_ID"
colnames(metadata)[1] <- "Sample_ID"

# Merge both the dataframe
data <- metadata %>% inner_join(abundance, by='Sample_ID')

# Check for the different conditions in the study_condition column
table(data$study_condition)
```

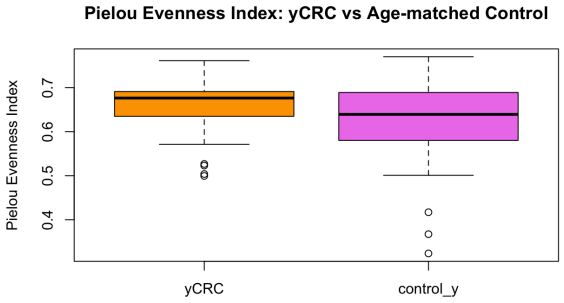
```
# Q2: How does Shannon Index and Pielou Evenness vary in yCRC and oCRC w.r.t age-matched controls

control <- data %>% filter(study_condition=="control")
# Filter the age-matched control samples as control_y (age<=50) and control_o (age>50) similar to filter for CRC samples

control_y <- data %>% filter(study_condition=='control') %>% filter(age<=50)
control_o <- data %>% filter(study_condition=='control') %>% filter(age>50)

# Obtained control_y (55 samples) and control_o (196 samples), total control = 251

# Calculate Shannon Index and Pielou Evenness for the control_y and control_o

control_y$shannon <- apply(control_y[, species_cols], 1, function(x){diversity(x, index = "shannon")})
control_y$richness <- apply(control_y[, species_cols], 1, function(x) {sum(x > 0)})
control_y$pielou <- with(control_y, ifelse(richness > 0, shannon / log(richness), NA))

control_o$shannon <- apply(control_o[, species_cols], 1, function(x){diversity(x, index = "shannon")})
control_o$richness <- apply(control_o[, species_cols], 1, function(x) {sum(x > 0)})
control_o$pielou <- with(control_o, ifelse(richness > 0, shannon / log(richness), NA))

# Boxplot for comparison between Shannon Index of yCRC vs Age-matched control (control_y) and oCRC vs Age-matched control (control_o)

boxplot(yCRC$shannon, control_y$shannon, col = c("red", "lightgreen"), names = c("yCRC", "control_y"),
        main = "Shannon Index: yCRC vs Age-matched Control", ylab = "Shannon Diversity Index")
boxplot(oCRC$shannon, control_o$shannon, col = c("yellow", "purple"), names = c("oCRC", "control_o"),
        main = "Shannon Index: oCRC vs Age-matched Control", ylab = "Shannon Diversity Index")

# Visualizing all Shannon Diversity Index in one plot
boxplot(yCRC$shannon, control_y$shannon,oCRC$shannon, control_o$shannon, col = c("red", "lightgreen", "yellow", "purple"),
        names = c("yCRC", "control_y", "oCRC", "control_o"), main = "Shannon Index: CRC vs Age-matched Control",
        ylab = "Shannon Diversity Index")
```

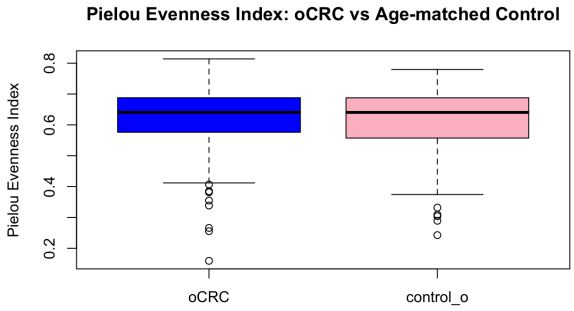
```r
# Perform t-test and Wilcoxon test for Shannon Index
shannon_y_con_ttest <- t.test(yCRC$shannon, control_y$shannon)
shannon_o_con_ttest <- t.test(oCRC$shannon, control_o$shannon)

shannon_y_con_wilcox <- wilcox.test(yCRC$shannon, control_y$shannon)
shannon_o_con_wilcox <- wilcox.test(oCRC$shannon, control_o$shannon)

print(shannon_y_con_ttest)
print(shannon_o_con_ttest)
print(shannon_y_con_wilcox)
print(shannon_o_con_wilcox)

# Boxplot for comparison between Pielou Evenness Index of yCRC vs Age-matched control (control_y) and oCRC vs Age-matched control

boxplot(yCRC$pielou, control_y$pielou, col = c("orange", "violet"), names = c("yCRC", "control_y"),
        main = "Pielou Evenness Index: yCRC vs Age-matched Control", ylab = "Pielou Evenness Index")
boxplot(oCRC$pielou, control_o$pielou, col = c("blue", "pink"), names = c("oCRC", "control_o"),
        main = "Pielou Evenness Index: oCRC vs Age-matched Control", ylab = "Pielou Evenness Index")

# Visualizing all Pielou Evenness in one plot
boxplot(yCRC$pielou, control_y$pielou, oCRC$pielou, control_o$pielou, col = c("orange", "violet", "blue", "pink"),
        names = c("yCRC", "control_y", "oCRC", "control_o"), main = "Pielou Evenness Index: CRC vs Age-matched Control",
        ylab = "Pielou Evenness Index")

# Perform t-test and Wilcoxon test for Pielou Evenness Index
pielou_y_con_ttest <- t.test(yCRC$pielou, control_y$pielou)
pielou_o_con_ttest <- t.test(oCRC$pielou, control_o$pielou)

pielou_y_con_wilcox <- wilcox.test(yCRC$pielou, control_y$pielou)
pielou_o_con_wilcox <- wilcox.test(oCRC$pielou, control_o$pielou)

print(pielou_y_con_ttest)
print(pielou_o_con_ttest)
print(pielou_y_con_wilcox)
print(pielou_o_con_wilcox)
```

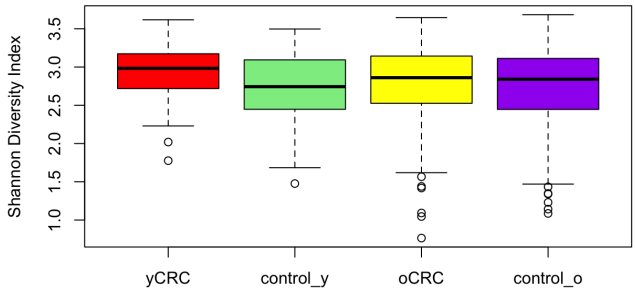
RESULTS OBTAINED:

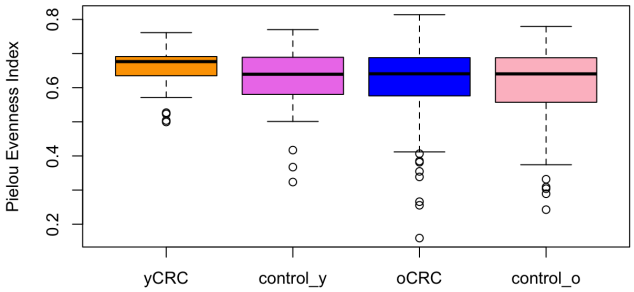












T-test and Wilcoxon Test Results:

Shannon Index

```
        Welch Two Sample t-test

data:  yCRC$shannon and control_y$shannon
t = 2.2289, df = 79.137, p-value = 0.02866
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 0.02279914 0.40335351
sample estimates:
mean of x mean of y
 2.924111  2.711035
```

```
        Welch Two Sample t-test

data:  oCRC$shannon and control_o$shannon
t = 0.9248, df = 409.07, p-value = 0.3556
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.05225818  0.14510909
sample estimates:
mean of x mean of y
 2.774724  2.728298
```

```
        Wilcoxon rank sum test with continuity correction

data:  yCRC$shannon and control_y$shannon
W = 1216, p-value = 0.03626
alternative hypothesis: true location shift is not equal to 0
```

```
        Wilcoxon rank sum test with continuity correction

data:  oCRC$shannon and control_o$shannon
W = 23132, p-value = 0.3017
alternative hypothesis: true location shift is not equal to 0
```

Pielou Evenness Index

```
        Welch Two Sample t-test

data:  yCRC$pielou and control_y$pielou
t = 1.8268, df = 85.565, p-value = 0.07122
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.00271273  0.06416842
sample estimates:
mean of x mean of y
0.6565381 0.6258103
```

```
        Welch Two Sample t-test

data:  oCRC$pielou and control_o$pielou
t = 0.60391, df = 410.19, p-value = 0.5462
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.01352047  0.02551171
sample estimates:
mean of x mean of y
0.6207132 0.6147175
```

```
        Wilcoxon rank sum test with continuity correction

data:  yCRC$pielou and control_y$pielou
W = 1171, p-value = 0.08515
alternative hypothesis: true location shift is not equal to 0
```

```
        Wilcoxon rank sum test with continuity correction

data:  oCRC$pielou and control_o$pielou
W = 22558, p-value = 0.5695
alternative hypothesis: true location shift is not equal to 0
```

INTERPRETATIONS & CONCLUSIONS:

1. T-test and Wilcoxon Test for Shannon Index: yCRC vs Age-matched control (control_y) shows **significant difference** (p-value <0.05). Thus they vary significantly, whereas **no significant variation** (p-value>0.05) is observed between Shannon Index: oCRC vs Age-matched control (contro_o)

2. T-test and Wilcoxon Test for Pielou Evenness Index: yCRC vs Age-matched control (control_y) as well as for Pielou Evenness Index: oCRC vs Age-matched control (control_o) shows **no significant difference** (p-value >0.05). Hence, they don't vary significantly.

Part C:Does age have an effect on the overall microbiome composition in patients with CRC?

```python
import pandas as pd
import numpy as np
import seaborn as sns
import matplotlib.pyplot as plt
from scipy.stats import pearsonr

# Loading  data from Excel
def load_data(path):
    meta_df = pd.read_excel(path, sheet_name='Metadata')
    micro_df = pd.read_excel(path, sheet_name='MicrobialAbundance')
    return meta_df, micro_df

# Computing  Shannon Index
def shannon_idx(abund):
    prop = abund / np.sum(abund)
    return -np.sum(prop * np.log(prop + 1e-10))

# Computing Pielou Evenness
def pielou_ev(abund):
    s = len(abund)
    h = shannon_idx(abund)
    return h / np.log(s)
```

```python
# Computing  microbiome indices
def calc_indices(micro_data):
    shannon_vals = micro_data.apply(shannon_idx, axis=1)
    pielou_vals = micro_data.apply(pielou_ev, axis=1)
    return shannon_vals, pielou_vals

# Computing Pearson correlation
def calc_corr(df):
    age_sh_corr, _ = pearsonr(df['age'], df['Shannon'])
    age_pi_corr, _ = pearsonr(df['age'], df['Pielou'])
    return age_sh_corr, age_pi_corr

# Plot Age vs Shannon Index
def plot_shannon(df):
    plt.figure(figsize=(10, 5))
    sns.scatterplot(data=df, x='age', y='Shannon', hue='study_condition', palette=['blue'])
    plt.title('Age vs Shannon Index in CRC Patients')
    plt.xlabel('Age')
    plt.ylabel('Shannon Index')
    plt.show()

# Plot Age vs Pielou Evenness
def plot_pielou(df):
    plt.figure(figsize=(10, 5))
    sns.scatterplot(data=df, x='age', y='Pielou', hue='study_condition', palette=['blue'])
    plt.title('Age vs Pielou Evenness in CRC Patients')
    plt.xlabel('Age')
    plt.ylabel('Pielou Evenness')
```

```
    plt.show()

# Main function to process data
def analyze_crc(path):
    meta_df, micro_df = load_data(path)
    micro_data = micro_df.drop(columns=['Unnamed: 0'], errors='ignore')

    if micro_data.isnull().any().any():
        print("Warning: Missing values found! Filling with 0.")
        micro_data = micro_data.fillna(0)

    shannon_vals, pielou_vals = calc_indices(micro_data)
    meta_df['Shannon'] = shannon_vals
    meta_df['Pielou'] = pielou_vals

    crc_df = meta_df[meta_df['study_condition'].isin(['yCRC', 'oCRC', 'CRC'])]
    age_sh_corr, age_pi_corr = calc_corr(crc_df)

    print(f"Age-Shannon Correlation: {age_sh_corr:.3f}")
    print(f"Age-Pielou Correlation: {age_pi_corr:.3f}")

    plot_shannon(crc_df)
    plot_pielou(crc_df)

# Runing analysis
file_path = 'YachidaCRC.xlsx'
analyze_crc(file_path)
```

Code explanation

a. Imports:
We use several libraries to help with this analysis. **pandas** is for loading and working with the data, **numpy** helps with numerical operations, **seaborn** and **matplotlib** are used for creating visualizations, and **scipy.stats** provides the function to calculate Pearson correlation.

b. Functions:

- The **load_data(path)** function loads the data from an Excel file, where we have two sheets: one for metadata and another for microbial abundance.

- **shannon_idx(abund)** calculates the Shannon Index, which gives a measure of microbial diversity based on the abundance of species.

- **pielou_ev(abund)** computes the Pielou Evenness, which tells us how evenly the species are distributed in the sample.
- **calc_indices(micro_data)** applies both the Shannon Index and Pielou Evenness calculations to each row of microbial data.
- **calc_corr(df)** calculates how strongly age is correlated with both the Shannon Index and Pielou Evenness using Pearson correlation.
- **plot_shannon(df)** creates a scatter plot to show how the Shannon Index varies with age.
- **plot_pielou(df)** generates a scatter plot to display how Pielou Evenness changes with age.

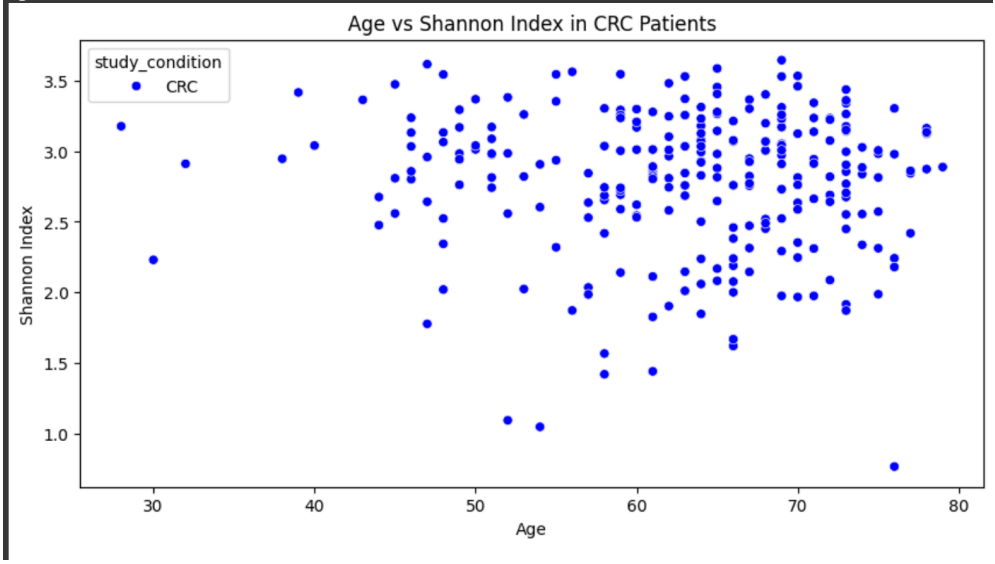
c. Main Function:
Finally, **analyze_crc(path)** is the main function that ties everything together. It loads the data, calculates the microbiome indices, calculates the correlations, and visualizes the results.

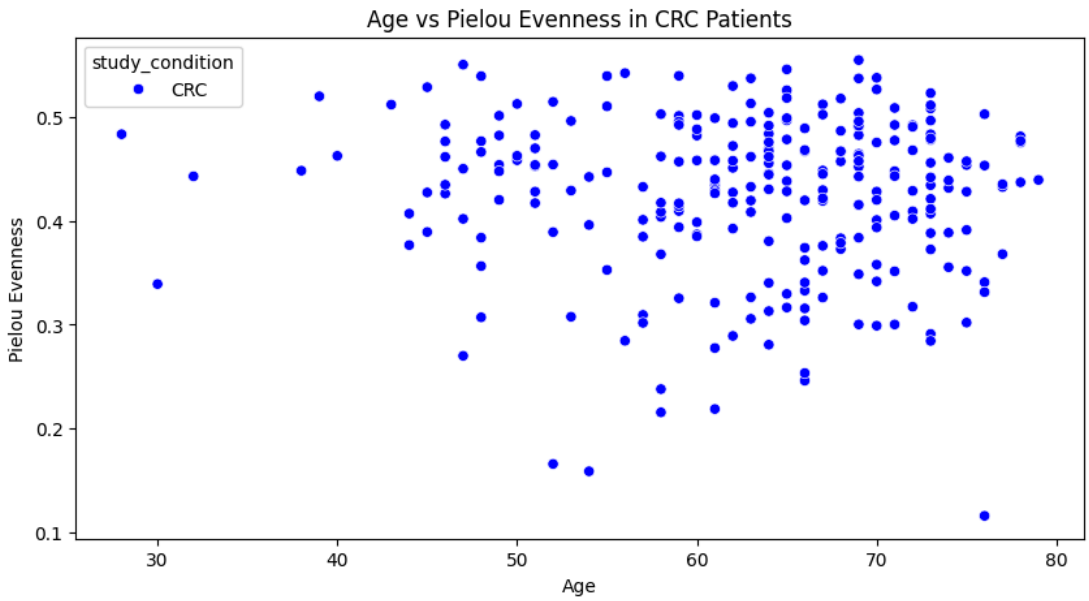
d. Analysis and Visualization:

After computing the indices, Shannon and Pielou values are added to the metadata. The data is then filtered to include only CRC cases ('yCRC', 'oCRC', or 'CRC'). The `calc_corr(df)` function calculates Pearson correlations between age and both indices to check for any relationship. Finally, `plot_shannon(df)` and `plot_pielou(df)` generate scatter plots to visualize how diversity and evenness vary with age, and the correlation values are printed.

Results:

```
Age-Shannon Correlation: -0.048
Age-Pielou Correlation: -0.048
```





Results and Interpretation

Pearson Correlation Analysis

- **Age vs. Shannon Index**:
  The Pearson correlation coefficient between age and the Shannon Index is **-0.048**, indicating a **very weak negative correlation**. This suggests that **age is not strongly associated with microbial diversity** in colorectal cancer (CRC) patients.

- **Age vs. Pielou Evenness**:
  Similarly, the Pearson correlation between age and Pielou Evenness is **-0.048**, showing an **equally weak negative correlation**. This implies that **age does not significantly influence the evenness** of microbial distribution in CRC patients.

Scatter Plot Interpretation

- **Age vs. Shannon Index**:
  The scatter plot depicting the relationship between age and the Shannon Index shows **no clear trend**. The data points are widely dispersed, further reinforcing the conclusion that **age has minimal impact on microbial diversity** in CRC patients.

- **Age vs. Pielou Evenness**:
  The scatter plot for age versus Pielou Evenness also demonstrates **no discernible pattern**. The spread of data points suggests that **age does not affect the uniformity** of the microbiome distribution.

Conclusion

Overall, the analysis indicates that **age does not have a significant effect** on either microbial diversity (as measured by the Shannon Index) or microbial evenness (as measured by Pielou Evenness) in CRC patients. Both correlation coefficients are close to zero, and visual inspection through scatter plots confirms the **absence of strong linear relationships**.

Part-D: Species that show differential age-associated changes in CRC patients vs control

Code:

```
import pandas as pd
import numpy as np
from scipy.stats import spearmanr
import matplotlib.pyplot as plt
import seaborn as sns

metadata = pd.read_csv('metadata.csv')
abundance = pd.read_csv('microbial_abundance.csv')

data = pd.merge(metadata, abundance, on='ID')

crc = data[data['study_condition'] == 'CRC'].copy()
control = data[data['study_condition'] == 'control'].copy()

species_cols = [col for col in abundance.columns if col != 'ID']

results = []

# Calculate Spearman correlation for each species
for species in species_cols:
    try:
        rho_crc, _ = spearmanr(crc['age'], crc[species])
        rho_ctrl, _ = spearmanr(control['age'], control[species])
        rho_diff = abs(rho_crc - rho_ctrl)
        results.append({
            'Species': species,
            'CRC_rho': rho_crc,
            'Control_rho': rho_ctrl,
            'Rho_diff': rho_diff
        })
    except:
        continue
correlation_df = pd.DataFrame(results)

# Filter significant differential species (|Δρ| > 0.4)
significant_species = correlation_df[correlation_df['Rho_diff'] > 0.4]

# Top 10 species by rho difference for analysis
top_10 = correlation_df.sort_values('Rho_diff', ascending=False).head(10)

# Display top 10 just for exploratory data analysis
print("\nTop 10 Species by Differential Age Correlation (CRC vs Control):\n")
print(top_10[['Species', 'CRC_rho', 'Control_rho', 'Rho_diff']].to_string(index=False))

print("\n" + "="*80)
print("Age-Associated Microbial Changes: CRC vs Control")
print("="*80)
print(f"Total microbial species tested: {len(correlation_df)}")
print(f"Threshold: |Δρ| > 0.4")

if not significant_species.empty:
    print(f"{len(significant_species)} species showed significant differential correlation
    print("These species exhibit age-associated changes that differ between CRC and contro
    for sp in significant_species['Species']:
        print(f"  • {sp}")
else:
    print("No species showed significant differential age-associated changes (Δρ > 0.4).")
    print("Age does not significantly influence species-specific microbial abundance in a
print("="*80)
```

Analysis:

Data Preparation
We merged patient metadata with microbial abundance.
We then divided the data into two groups: CRC patients and healthy controls.

Correlation Analysis
For each microbial species, we computed the Spearman correlation (ρ) between species abundance and age separately in CRC and control groups.
We calculated the absolute difference in these correlations:

$$|\Delta\rho| = |\rho CRC - \rho Control|$$

Threshold for Significance
Species with |Δρ| > 0.4 were considered to have meaningful differential age-association.

Main Results

- Total species tested: 717
- Threshold used: |Δρ| > 0.4
- No species showed |Δρ| > 0.4

Conclusion

No microbial species showed significant differential age-associated changes between CRC and control groups.
This suggests that age does not significantly influence microbial abundance in a way that distinguishes CRC from healthy individuals.

```
Top 10 Species by Differential Age Correlation (CRC vs Control):

                     Species   CRC_rho  Control_rho  Rho_diff
         Klebsiella_variicola -0.033988     0.213577  0.247566
           Bacteroides_clarus  0.107333    -0.138836  0.246169
          Murimonas_intestini  0.130126    -0.105663  0.235790
    Raoultella_ornithinolytica -0.108634     0.122026  0.230661
            Gemmiger_sp_An194  0.134261    -0.095770  0.230031
     Parabacteroides_johnsonii  0.111422    -0.112567  0.223989
          Megasphaera_elsdenii -0.246532    -0.023969  0.222563
Actinomyces_sp_oral_taxon_170  0.099328    -0.120603  0.219931
Ruminococcaceae_bacterium_D16  0.123581    -0.082995  0.206576
      Veillonella_tobetsuensis -0.063242     0.141886  0.205128

================================================================================
Age-Associated Microbial Changes: CRC vs Control
================================================================================
Total microbial species tested: 717
Threshold: |Δρ| > 0.4
No species showed significant differential age-associated changes (Δρ > 0.4).
Age does not significantly influence species-specific microbial abundance in a way that differentiates CRC patients from healthy individuals
================================================================================
```