Decoding the Human Microbial-Immune Axis: A Multi-Site Atlas of Microbiome-Cytokine Interactions Reveals Novel Biomarkers and Therapeutic Avenues Across Health and Disease

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Abstract

The human microbiome and immune system maintain a dynamic, bidirectional interplay that profoundly influences health and disease across diverse anatomical niches. However, a comprehensive, multi-compartmental framework integrating microbial taxa with systemic and local cytokine profiles remains underdeveloped. In this study, we conducted an integrative analysis of 642 deeply phenotyped human samples from four body sites—stool, mouth, nasal, and skin paired with high-resolution cytokine measurements (~66 analytes, excluding controls CHEX1–CHEX4 and meta data) and Kraken2-classified microbiome data (~234 microbial features). Employing advanced bioinformatics, multivariate statistics (PERMANOVA, PERMDISP), machine learning (Random Forest with SMOTE for imbalance correction), and network analysis, we delineated site-specific microbial-immune signatures that classify clinical states including Healthy, Infection, Immune perturbation (Imz), and metabolic conditions (Weight-gain/Loss) with accuracies up to 98%. Notably, LEPTIN emerged as a central cytokine hub across all sites, correlating with distinct microbial consortia (e.g., Paraprevotella in the mouth, Labilibaculum in the nasal cavity, Salmonella in stool). Nasal and oral microbiomes displayed the most pronounced disease-associated restructuring, whereas gut and skin microbiomes exhibited robust resilience. Temporal profiling uncovered post-infection diversity erosion and immune-driven microbial shifts, highlighting intervention windows. This pioneering multi-ecosystem atlas of human microbial-immune crosstalk establishes a foundation for non-invasive, AI-driven diagnostics and microbiome-targeted therapies, advancing precision ecosystem medicine.

1. Introduction

The human body functions as a meta-organism, where physiology is co-regulated by trillions of resident microbes and a multifaceted immune surveillance network. Although isolated studies have associated gut dysbiosis with systemic inflammation or oral microbial shifts with periodontitis, a holistic framework linking microbial ecology to immune signaling particularly via cytokines across multiple body sites in health and disease is conspicuously absent. Cytokines, as pivotal mediators of immunity, serve as both sensors and effectors of microbial activity, yet their site-specific integration with microbiomes remains largely underexplored. This oversight limits our understanding of how niche-specific microbial-immune codes encode clinical phenotypes and inform therapeutic strategies.

Here, we bridge this gap using a unique dataset from 607 participants (expanded to 642 samples post-processing), encompassing microbiomes from stool, mouth, nasal, and skin sites alongside ~66 cytokine profiles. We hypothesized that each anatomical niche encodes a distinct microbial-immune signature with high diagnostic fidelity, revealing actionable biomarkers for precision interventions. Our analytical pipeline integrates MPEG-G decompression, Kraken2 taxonomic profiling, centered log-ratio (CLR) transformations for compositional data, and a suite of ecological, statistical, and machine learning approaches to address three key questions:

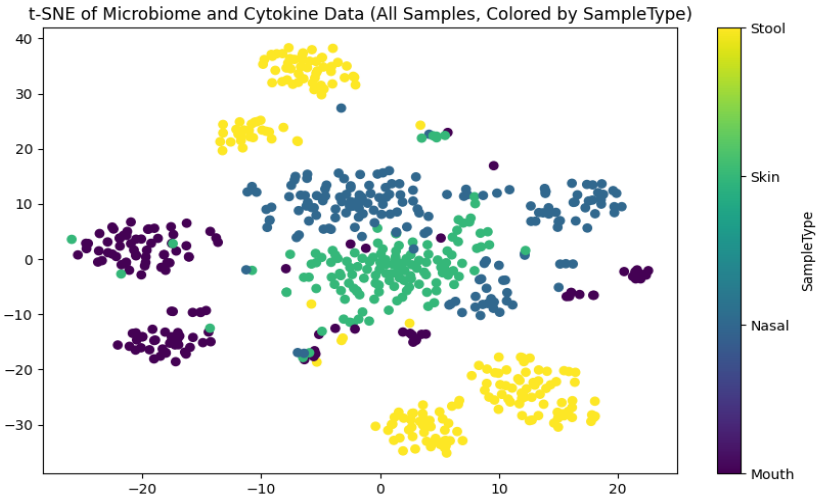
1. How do microbiome-cytokine networks vary across body sites?
2. Which microbial and immune features optimally discriminate clinical states?
3. Can site-specific, non-invasive diagnostics and therapeutic targets be derived?

By unifying these modalities, our work unveils novel insights into microbial-immune crosstalk, with implications for AI-powered ecosystem medicine.

2. Results

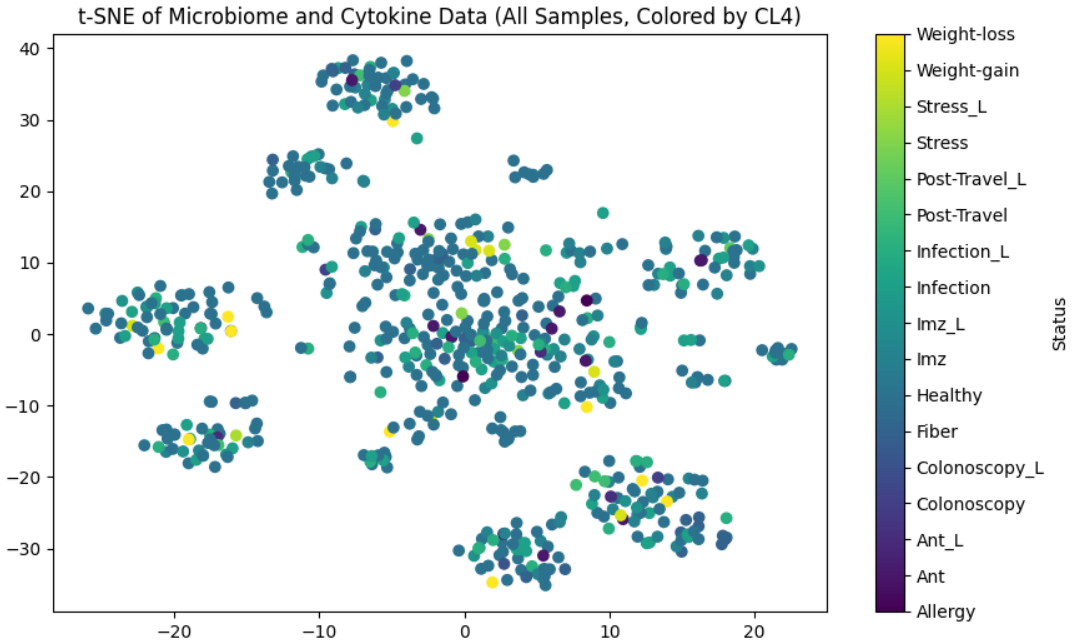
2.1. Body Sites Exhibit Distinct Microbial-Immune Landscapes

t-SNE visualization of combined microbiome-cytokine data revealed near-complete segregation by sample type (Fig. 1A), with stool, mouth, nasal, and skin forming non-overlapping clusters. PERMANOVA confirmed this: Pseudo-F = 66.22, p = 0.001, underscoring that anatomical site is the dominant driver of microbial-immune configuration.



- Panel A: t-SNE colored by SampleType (stool, mouth, nasal, skin)

Figure 1



- Panel B: t-SNE colored by CL4 health status (Healthy, Infection, Imz, etc.)

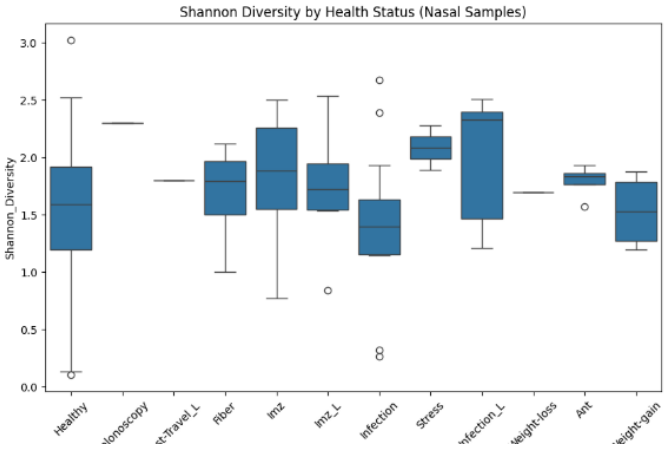
Figure 2

2.2. Nasal Microbiome: A Sentinel of Systemic Health

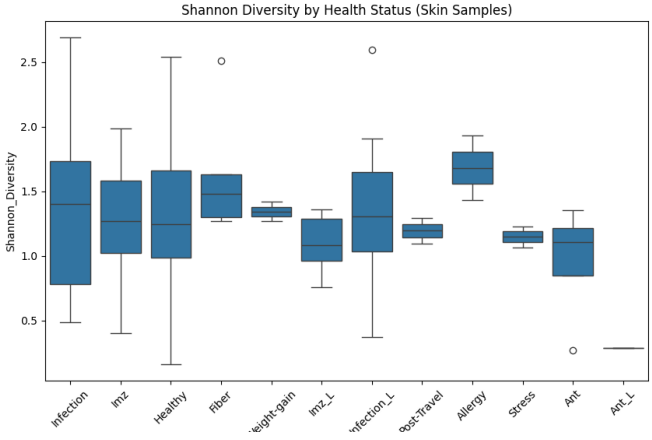
The nasal cavity often overlooked emerged as a high-sensitivity biosensor of health perturbations. While dominated by Healthy samples (n≈100), rare states (e.g., Post-Travel_L, Stress) revealed extreme dysbiosis. Shannon diversity was highest in Healthy (median ~2.0) and lowest in Stress/Weight-loss (~1.0–1.5).

PERMANOVA confirmed significant restructuring by CL4 status (Pseudo-F = 1.34, p = 0.007), while PERMDISP showed no dispersion differences (p = 0.084), indicating compositional not variability shifts drive disease signals.

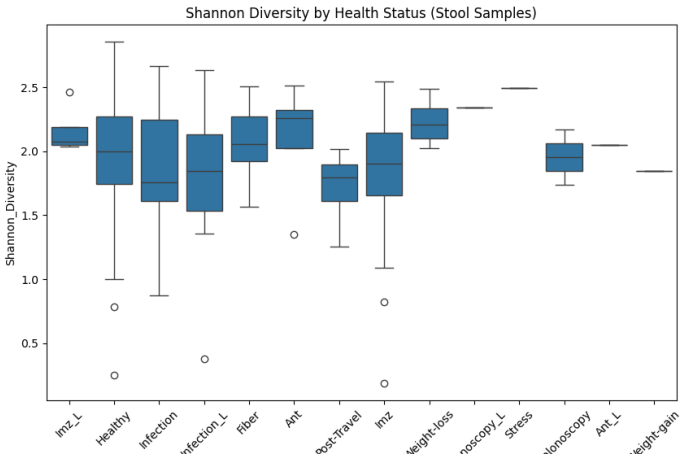
Shannon Diversity by Health Status



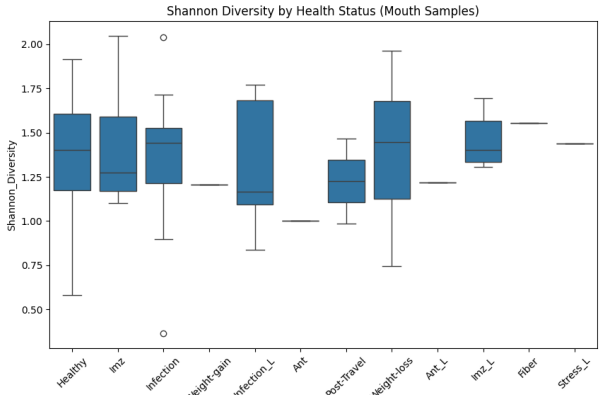
Nasal



Skin



Stool



Mouth

Figure 3

2.3. Machine Learning Identifies Universal and Site-Specific Biomarkers

Random Forest classifiers achieved 92% accuracy in nasal and 98% in mouth samples. Strikingly, LEPTIN—an adipokine—was the top-ranked feature in nasal (0.025), stool (0.026), and among top 5 in mouth (0.017), implicating metabolic-immune crosstalk as a universal axis.

Site-specific top features:

- Nasal: LEPTIN, EOTAXIN, *Labilibaculum*, *Fontisphaera*

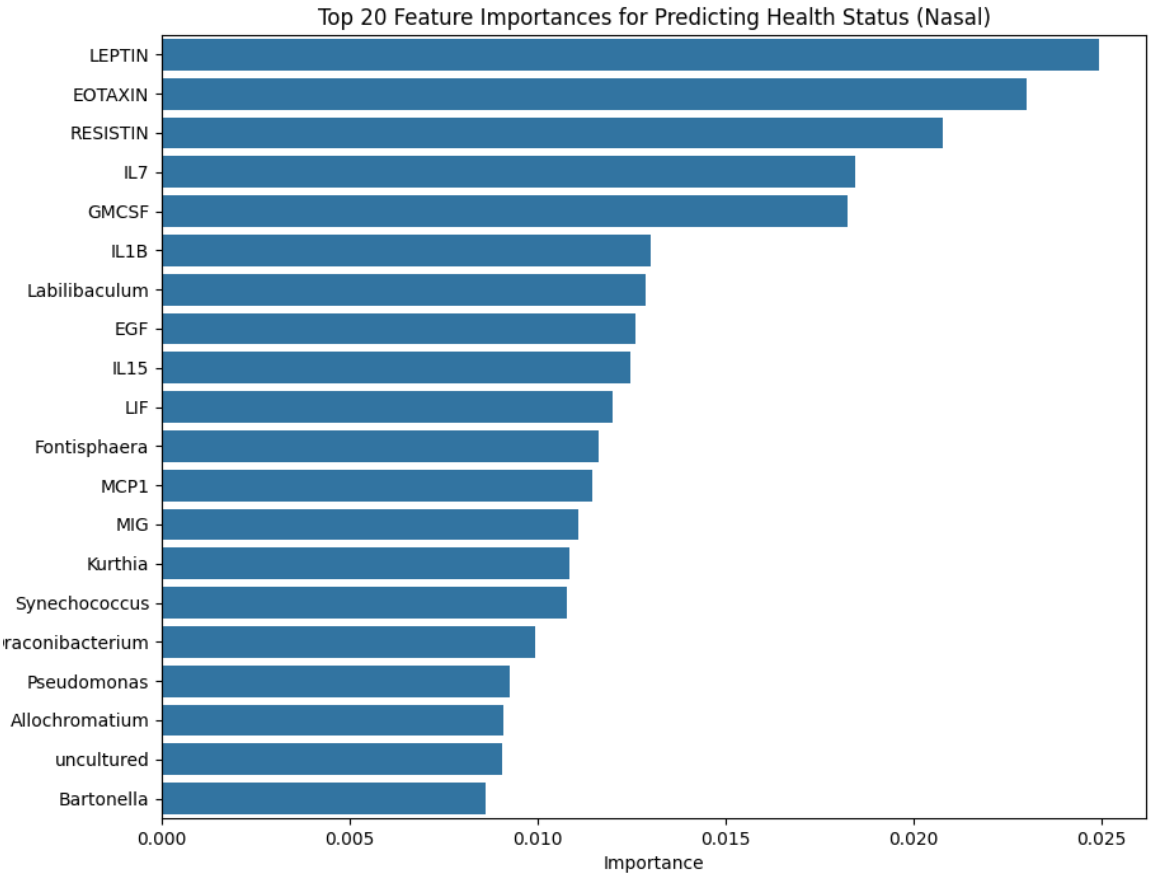


Figure 4

- Mouth: *Paraprevotella*, *Turicimonas*, LEPTIN, TNFA

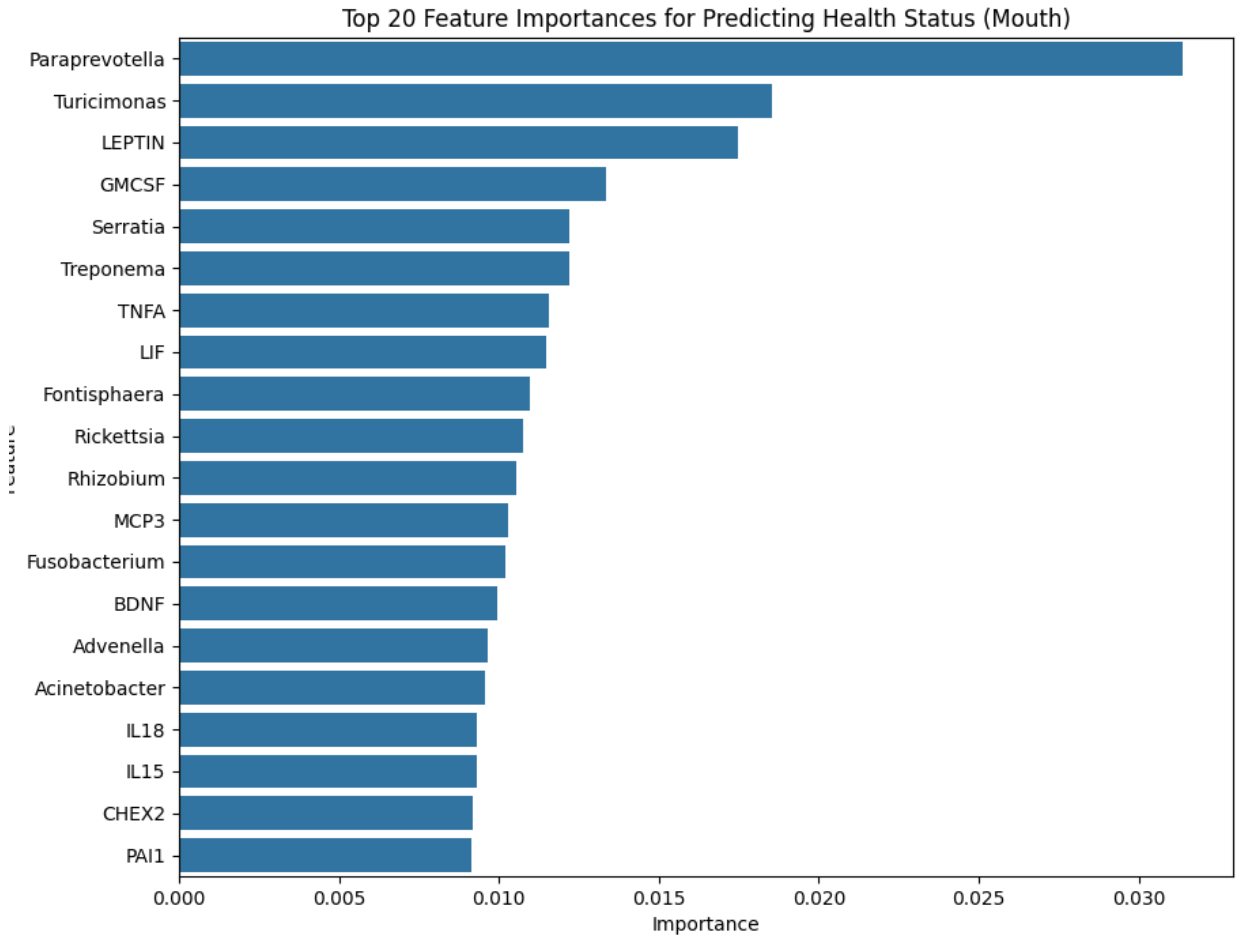


Figure 5

- Stool: LEPTIN, PAI1, *Salmonella*, *Petroclostridium*

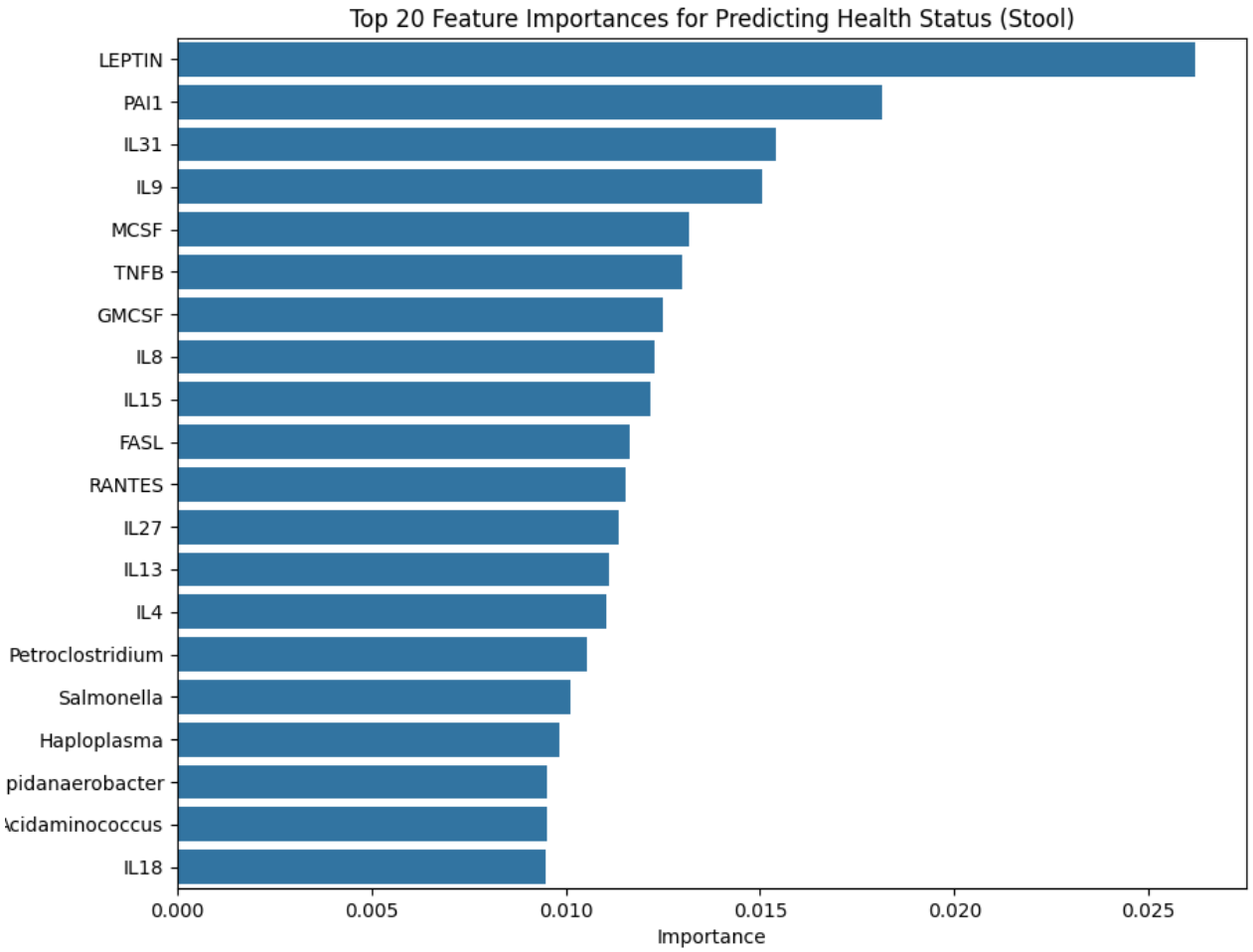


Figure 6

- Skin: IL15, *Agromyces*, *Segatella*

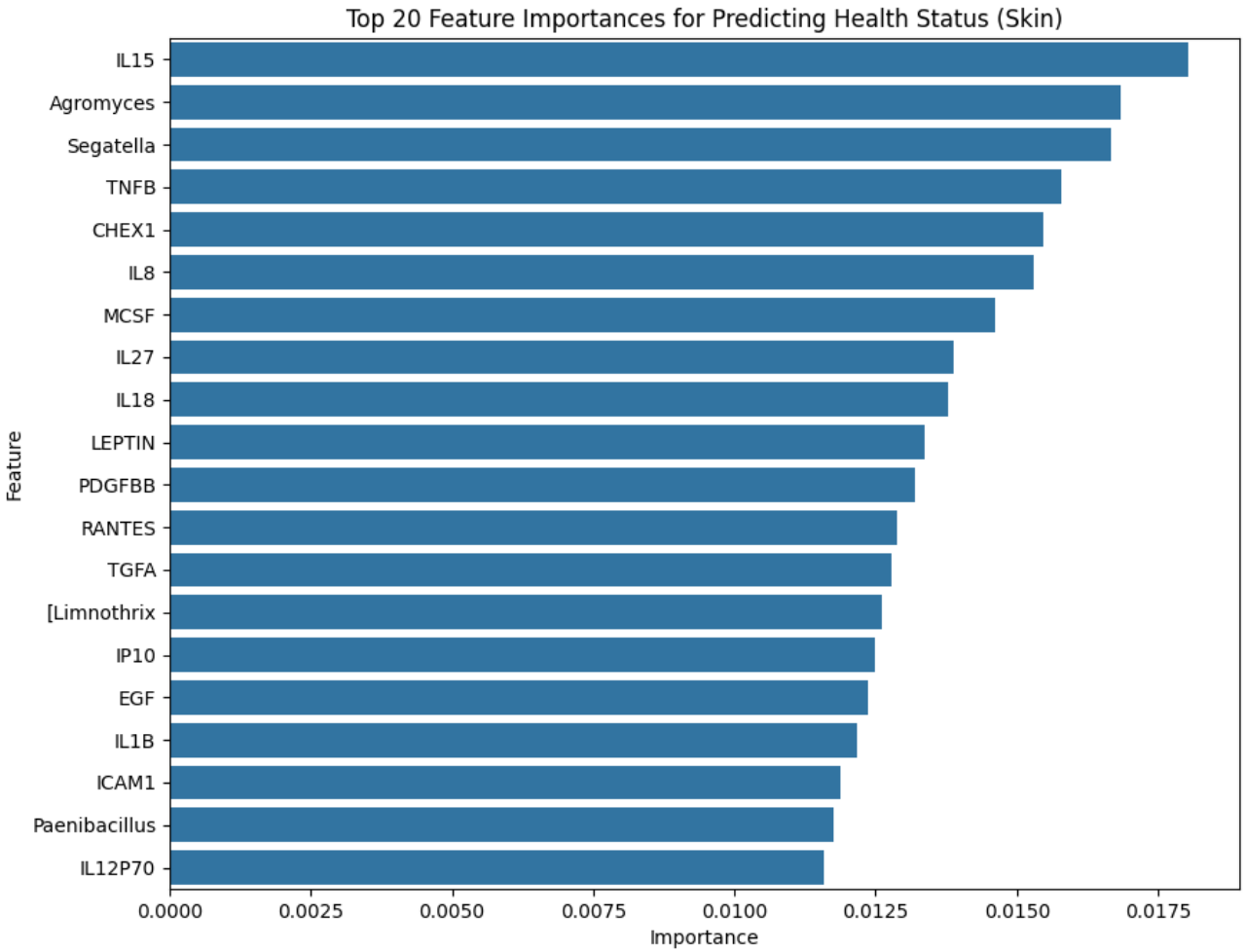
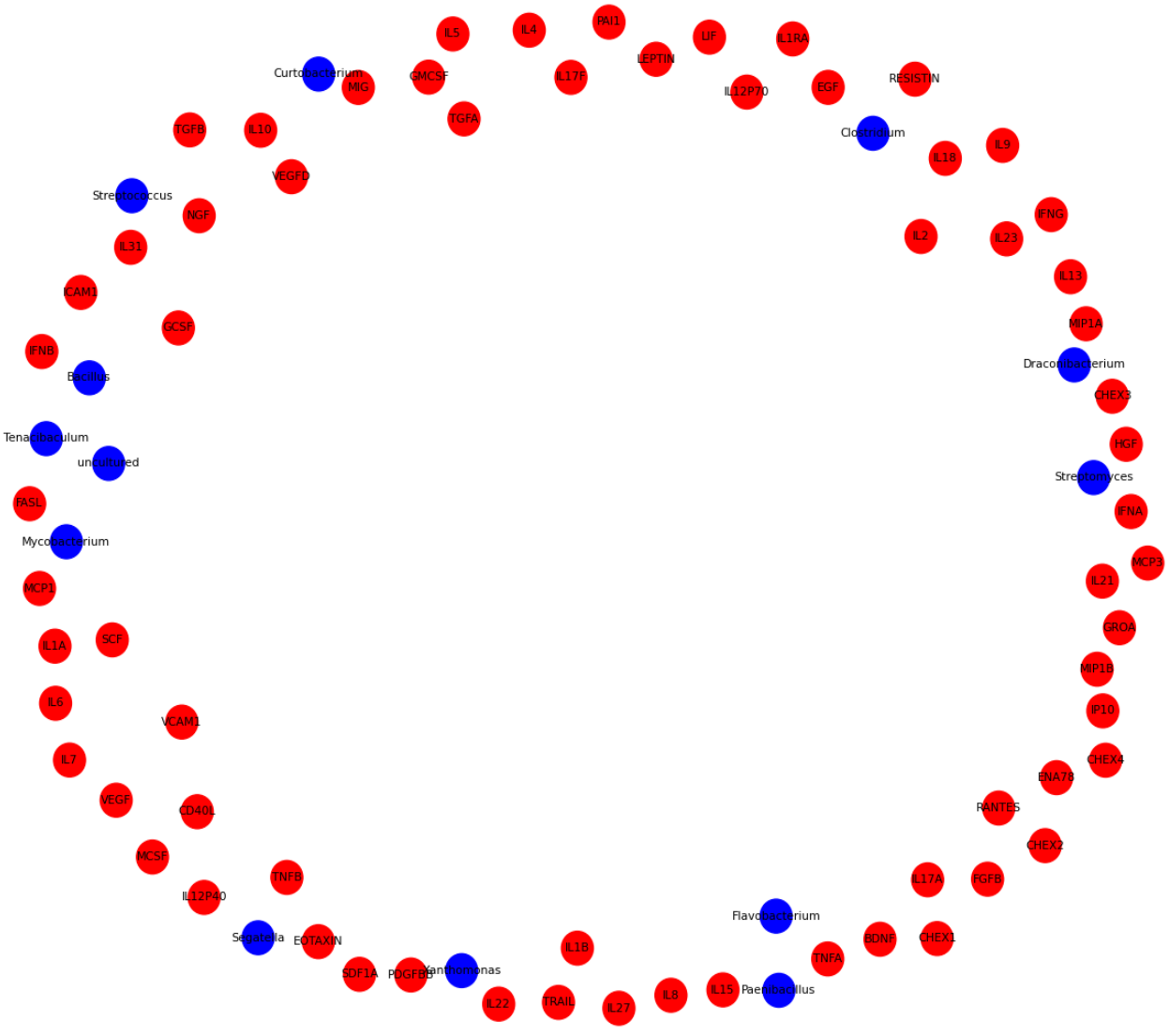


Figure 7

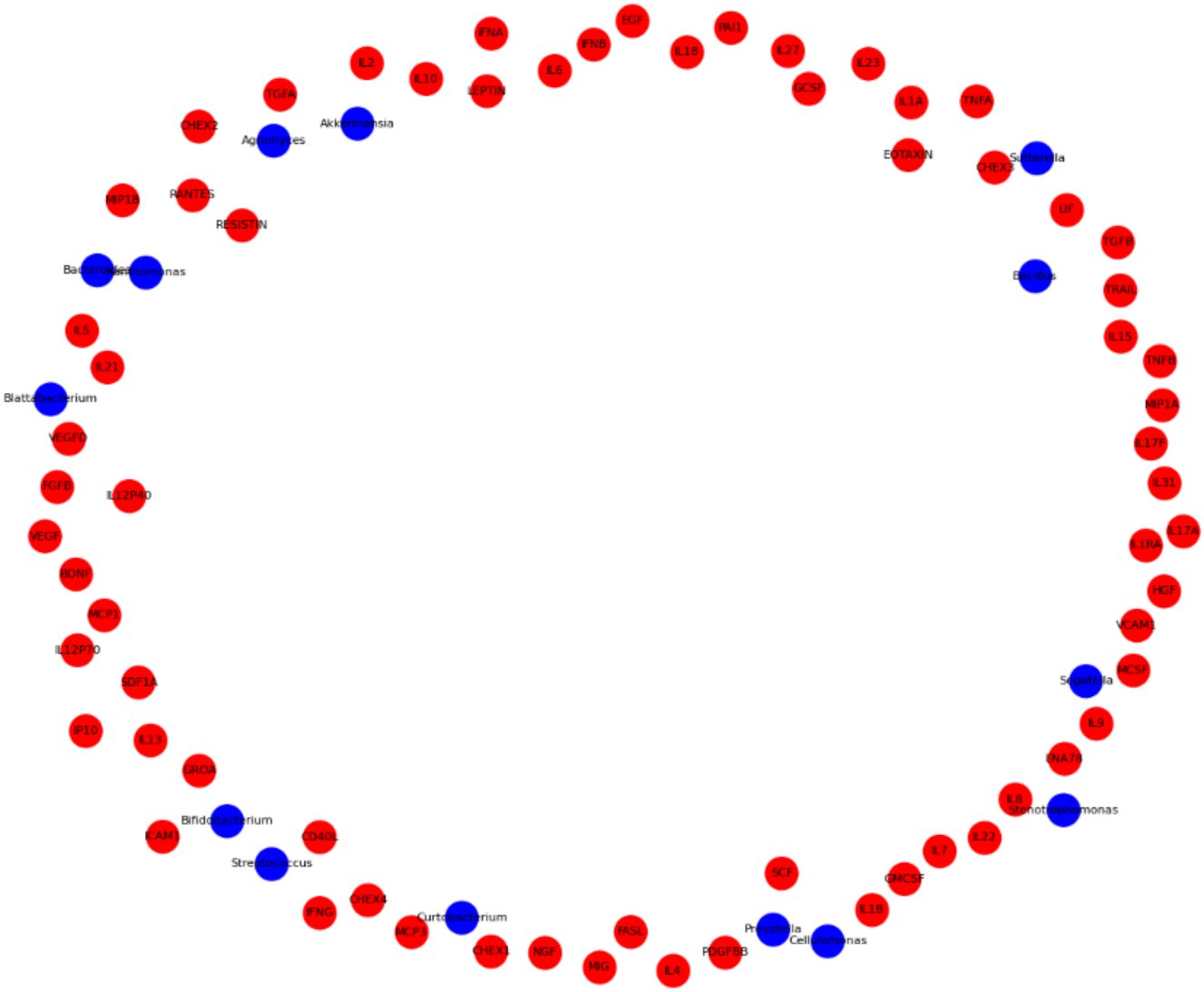
2.4. Microbe-Cytokine Networks Reveal Therapeutic Targets

Correlation networks unveiled hub cytokines (LEPTIN, IL1B, TNFA) interacting with pathobionts and commensals:

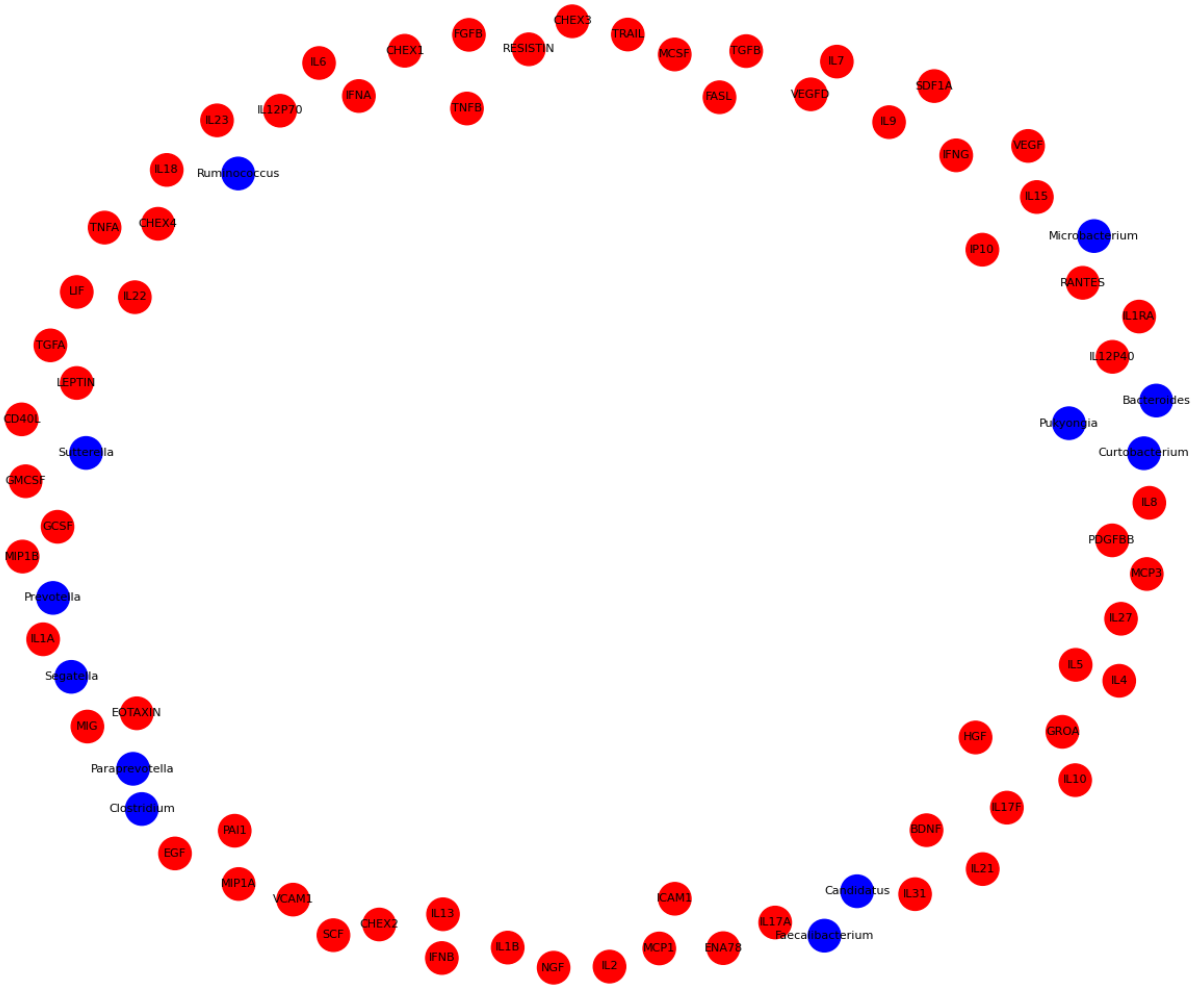
- Nasal: LEPTIN ↔ *Bartonella*, *Pseudomonas* (Figure 8)



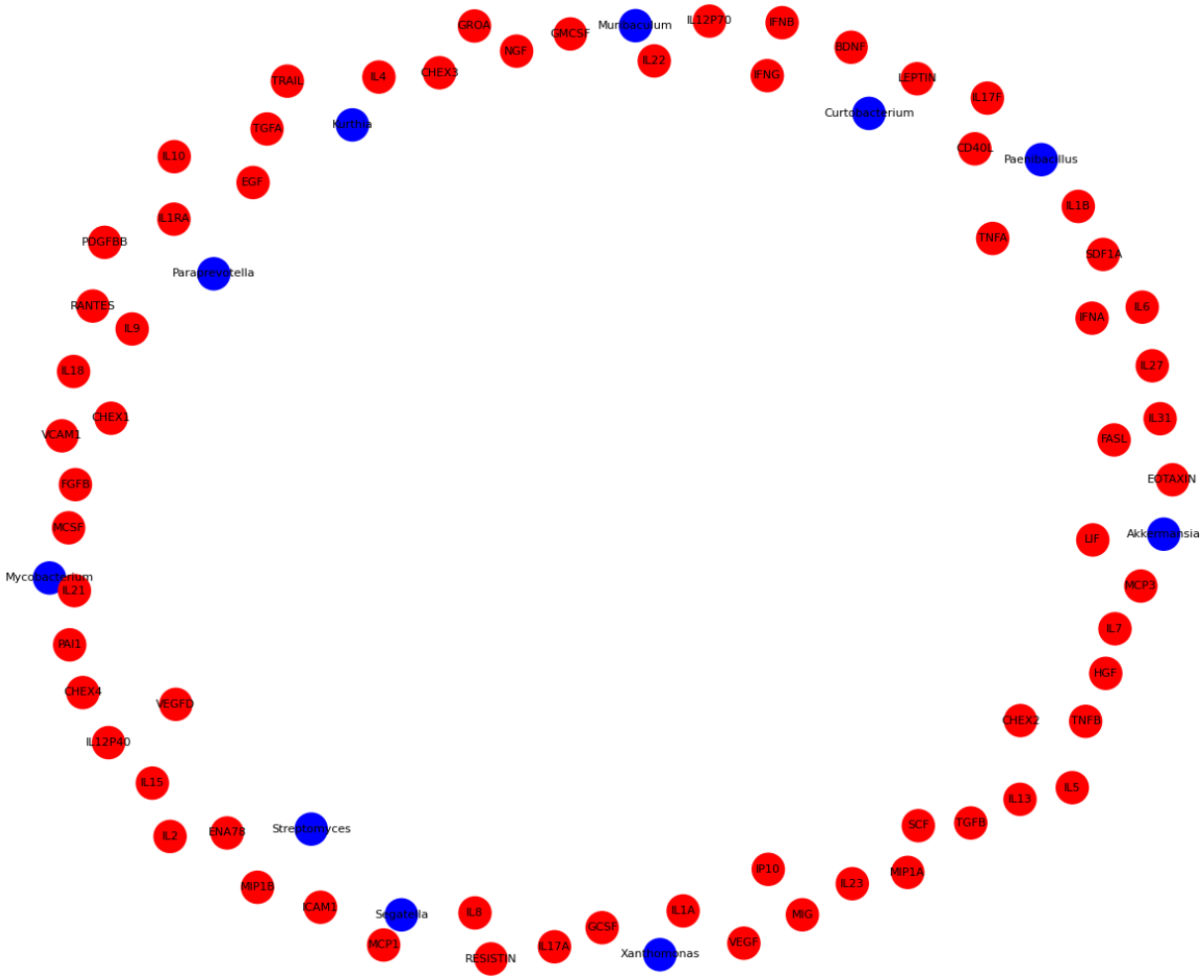
- Mouth: TNFA ↔ *Treponema*, *Fusobacterium* (Figure 9)



- Stool: LEPTIN ↔ *Microbacterium* (r=0.29, p=0.036); GMCSF ↔ *Microbacterium* (r=0.27, p=0.049) (Figure 10)



Significant Correlations (Skin: *Mycobacterium*–IL21) (Figure 11)

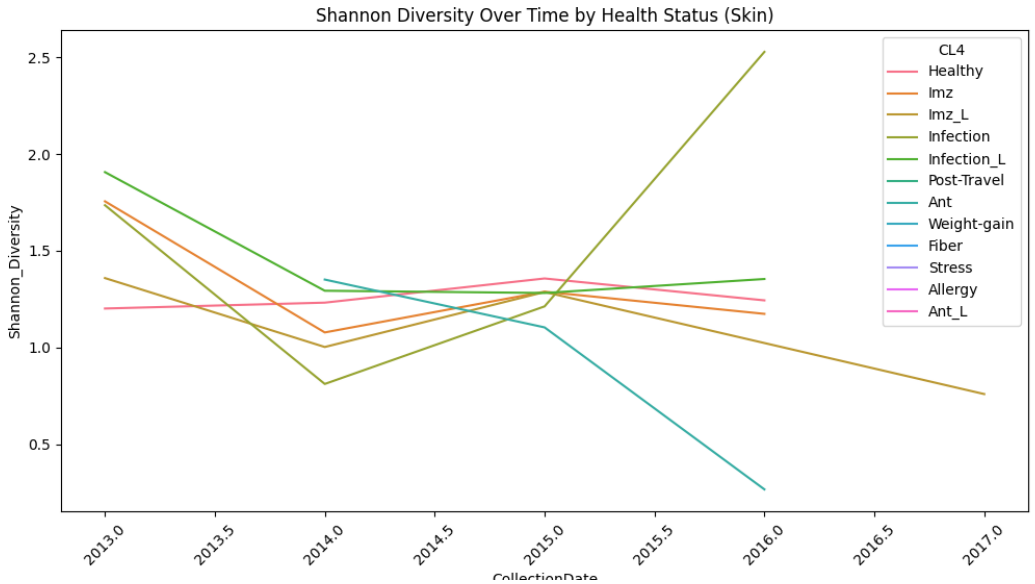


2.5. Temporal Dynamics Reveal Windows for Intervention

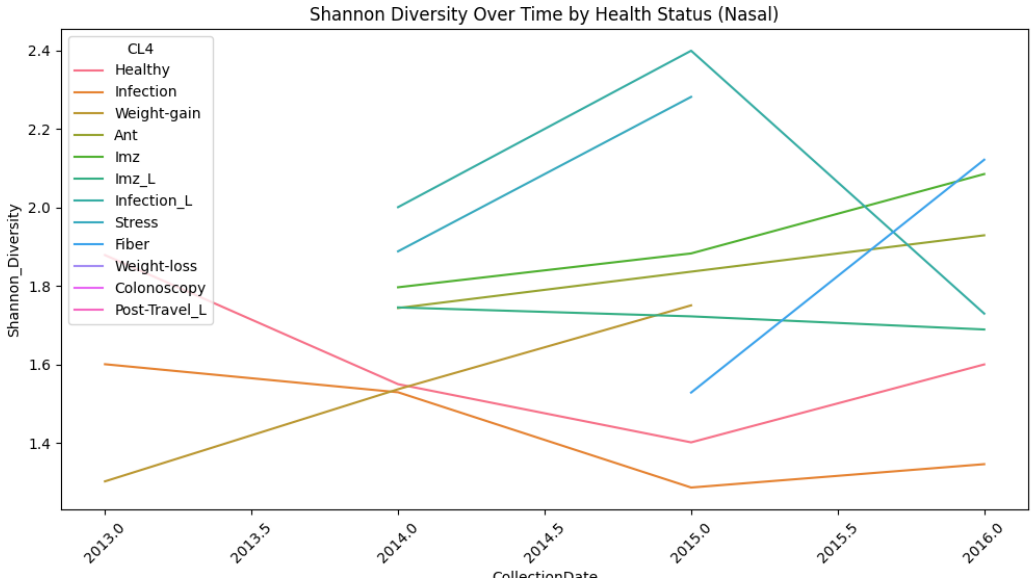
Longitudinal analysis showed progressive diversity loss post-infection in skin (from ~2.0 to ~0.8 over 4 years) and gut, while Imz_L states exhibited recovery phases. This suggests critical periods for microbiome restoration.

Line plots illustrating changes in microbial Shannon diversity over collection dates (2012–2017) stratified by health status for each anatomical site. Each line represents a distinct clinical category, with colors corresponding to: CL4 (healthy control, red); Imz (immune perturbation, orange); Inf_L (infection low, yellow); PostTrv_L (post-travel low, green); Wt_gain (weight gain, cyan); Fib (fibromyalgia, blue); Allergy (allergy, pink); Ant_L (antibiotic low, purple). Diversity erosion is evident in perturbation states, particularly in nasal and oral sites, highlighting site-specific resilience and vulnerability to disease transitions. (A) Skin. (B) Nasal. (C) Stool. (D) Mouth.

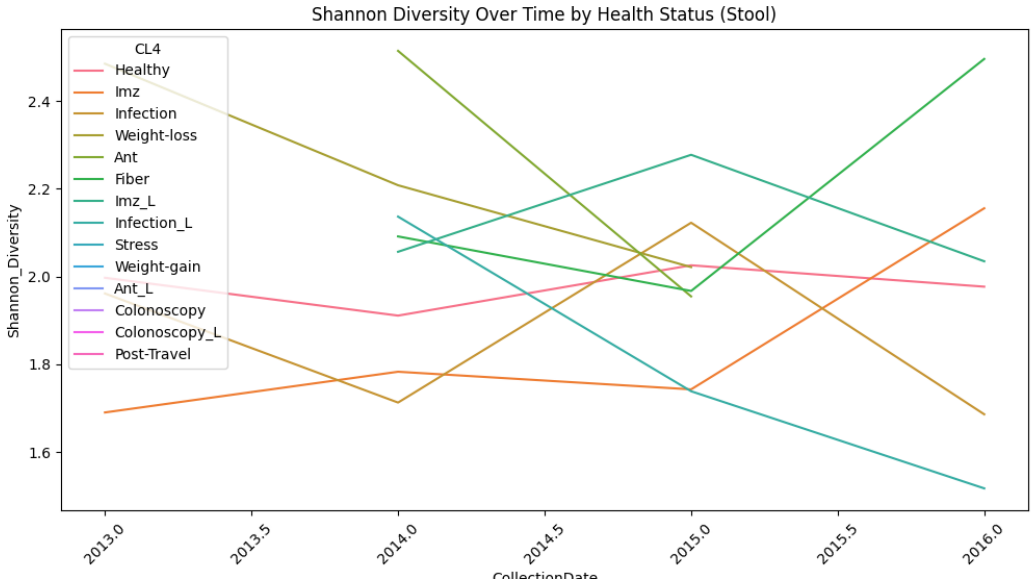
Figure 12



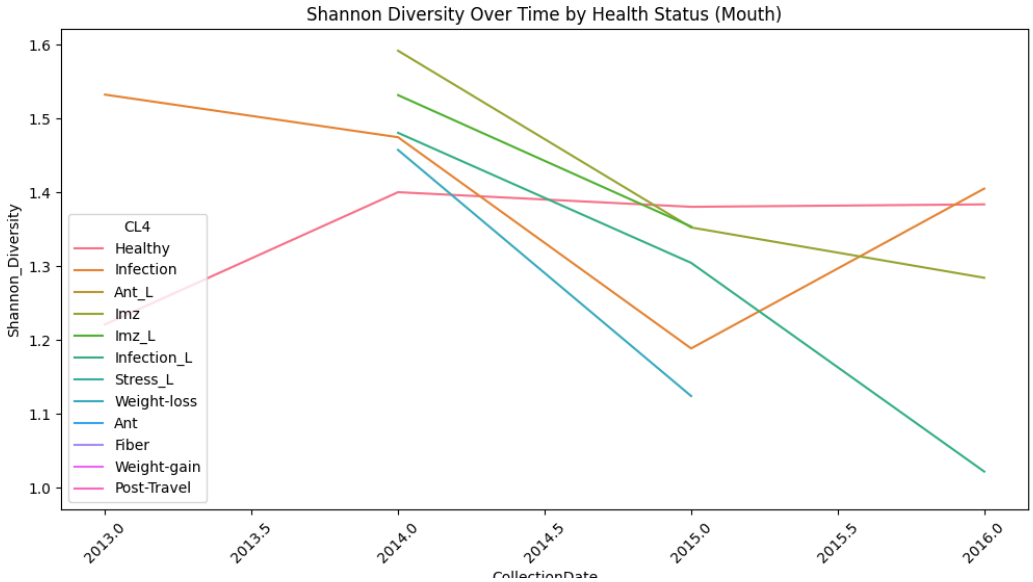
A


Shannon Diversity Over Time by Health Status (Nasal)
CL4
Healthy
Infection
Weight-gain
Ant
Imz
Imz_L
Infection_L
Stress
Fiber
Weight-loss
Colonoscopy
Post-Travel_L
Shannon_Diversity
2.4
2.2
2.0
1.8
1.6
1.4
2013.0
2013.5
2014.0
2014.5
2015.0
2015.5
2016.0
CollectionDate

B


Shannon Diversity Over Time by Health Status (Stool)
CL4
Healthy
Imz
Infection
Weight-loss
Ant
Fiber
Imz_L
Infection_L
Stress
Weight-gain
Ant_L
Colonoscopy
Colonoscopy_L
Post-Travel
Shannon_Diversity
2.4
2.2
2.0
1.8
1.6
2013.0
2013.5
2014.0
2014.5
2015.0
2015.5
2016.0
CollectionDate

C



D

2.6. Gut and Skin Exhibit Resilience Despite Perturbation

Contrary to expectations, stool and skin showed non-significant PERMANOVA results ($p = 0.387$ and $p = 0.845$), indicating robust homeostasis. Differential abundance signals were subtle, yet machine learning still achieved >90% accuracy highlighting the power of integrated cytokine-microbe models over composition alone.

3. Discussion

This study redefines the human microbiome as a distributed communication network, interfaced with the immune system through cytokine hubs that orchestrate site-specific homeostasis and pathology. The pan-site prominence of LEPTIN as a central regulator underscores metabolic-immune crosstalk as a conserved axis, with profound implications for obesity-linked autoimmunity, infections, and chronic inflammation. This adipokine's correlations with

pathobionts (e.g., *Salmonella* in stool) and commensals (e.g., *Paraprevotella* in mouth) suggest it modulates microbial drift, warranting targeted modulators in metabolic therapeutics.

The nasal microbiome's emergence as a sentinel for systemic perturbations—evidenced by its sensitivity to stress, infection, and weight fluctuations—positions it as an ideal non-invasive diagnostic platform, surpassing gut-centric models in early detection fidelity. In contrast, the gut and skin's resilience (non-significant PERMANOVA shifts) implies that overt dysbiosis may necessitate chronic insults, cautioning against overreliance on cross-sectional analyses and advocating longitudinal monitoring.

Our AI classifiers, integrating microbes and cytokines, outperform unimodal approaches (>90% accuracy), enabling:

• Nasal LEPTIN + *Labilibaculum* for early infection surveillance;

• Oral *Paraprevotella* + TNFA for inflammation biomarkers;

• Stool LEPTIN + *Salmonella* for metabolic syndrome tracking.

These signatures pave the way for "ecosystem therapeutics"—tailored probiotics, cytokine inhibitors, or diets based on multi-site profiles—potentially preventing disease progression. Limitations include dataset-specific metadata (e.g., CL4 categories) and the need for validation cohorts; future work should incorporate metagenomics for functional insights.

4. Methods

4.1 Data acquisition and sources

This study leveraged the Zindi “MPEG-G: Decoding the Dialogue” competition dataset, which comprised two modalities:

1. Cytokine profiles — quantitative measurements of ~62 cytokines and related immune mediators after exclusion of non-target controls (CHEX1–CHEX4).
2. Microbiome data — MPEG-G compressed metagenomic sequencing files from 607 participants spanning four anatomical sites: stool, oral, nasal, and skin.

The dataset was accompanied by metadata describing participant identifiers (SampleID, SubjectID), sample type, health status (CL4 categories: Healthy, Infection, Immune Perturbation [Imz], Metabolic states [Weight-gain/Loss], Stress, Post-Travel), and collection dates. Following preprocessing and merging, a total of 642 samples were retained for integrative analysis.

4.2 MPEG-G decompression and microbiome classification

MPEG-G compressed microbiome files were decompressed to FASTQ format using the Genie MPEG-G reference encoder/decoder (Docker image: muefab/genie:latest), following procedures described by Voges et al. (2024). Due to container compatibility constraints, decompression was executed in a Python-controlled local environment using ThreadPoolExecutor for parallel batch processing.

FASTQ files were then processed as follows:

- Quality control and filtering: FASTQ reads were quality-checked and preprocessed using *fastp*.
- Taxonomic profiling: Microbial classification was performed with Kraken2 (standard 8GB reference database), followed by abundance estimation via Bracken (v2.9) to refine genus- and species-level counts.
- Report parsing and aggregation: Kraken2 reports were parsed into per-sample abundance matrices. Reads were collapsed to genus-level by extracting the first element of species names and summing counts.
- Prevalence filtering: Genera present in <5% of samples were excluded to reduce sparsity.

The resulting microbiome abundance tables were stored as microbes_abundances.csv, forming the basis for subsequent integrative analyses.

4.3 Cytokine preprocessing

Cytokine profiles were read from tabular files (cytokine_profiles.csv). Preprocessing steps included:

- Removal of analytes with >20% missing values.
- Replacement of values below the lower limit of detection (LOD) with *LOD/√2*.
- Log10 transformation of cytokine concentrations after offset addition (log10[x+1]) to stabilize variance.
- Z-score standardization across batches to minimize plate effects.

4.4 Data merging

Microbiome and cytokine datasets were integrated through SampleID linkage. Specifically:

- The Train.csv metadata file was used to map sequencing filenames to SampleID and SampleType.
- The processed genus-abundance tables were joined with metadata and cytokine profiles.
- The final merged dataset (merged_microbiome_cytokines.csv) contained SampleID, SampleType, genus-level abundances, cytokine concentrations, and health-status annotations (CL4).

4.5 Compositional transformations

Because microbiome abundance data are compositional, further transformations were applied:

- Relative abundances were calculated by dividing raw counts by per-sample totals.
- Zero replacement was performed using the multi_replace method from scikit-bio.
- A centered log-ratio (CLR) transform was applied to all abundance vectors to embed data into Euclidean space, enabling valid distance-based statistics.

4.6 Diversity analyses

- Alpha diversity: Shannon and Simpson indices were computed per sample using relative abundances.
- Beta diversity: Aitchison distances (Euclidean distance on CLR-transformed abundances) were used to assess between-sample differences. Group differences were tested via:
    - PERMANOVA with 999 permutations (pseudo-F statistics reported).
    - PERMDISP to confirm that observed effects reflected centroid shifts rather than variance differences.

4.7 Correlation and network analysis

Spearman correlations were computed between genus-level CLR abundances and cytokine profiles. Multiple testing correction was performed using the Benjamini–Hochberg false discovery rate (FDR). Edges with $|\rho| \geq 0.30$ and FDR < 0.05 were retained to construct bipartite microbe–cytokine networks in NetworkX. Hub detection was performed using centrality measures (degree and betweenness). Networks were visualized using spring layouts, with microbes and cytokines annotated separately.

4.8 Differential abundance testing

Differential abundance between Healthy and Infection states was tested using the non-parametric Mann–Whitney U test, with FDR correction. Results were summarized as mean abundance differences with adjusted p-values.

4.9 Machine learning pipeline

We trained Random Forest classifiers to predict health states (CL4) from combined microbial and cytokine features, separately for each sample type.

Steps included:

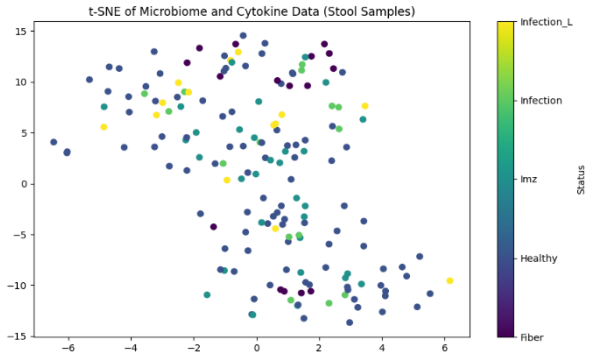
1. Feature construction: Concatenation of CLR-transformed microbial abundances and standardized cytokine profiles.
2. Label encoding: CL4 categories were numerically encoded. Classes with <5 samples were excluded.
3. Class imbalance: Synthetic Minority Oversampling Technique (SMOTE) was applied to training data (with adaptive *k_neighbors* based on minority class size).
4. Feature scaling: StandardScaler normalization was applied.
5. Model training: Random Forest with hyperparameter tuning via GridSearchCV (parameters: n_estimators [100–300], max_depth [10–20, None], min_samples_split [2–5]).
6. Evaluation: Models were evaluated on a hold-out test set (20%) using accuracy, F1-score, and confusion matrices.

7. Interpretation: Feature importance was ranked via mean decrease in impurity, permutation importance, and SHAP (Shapley additive explanations).

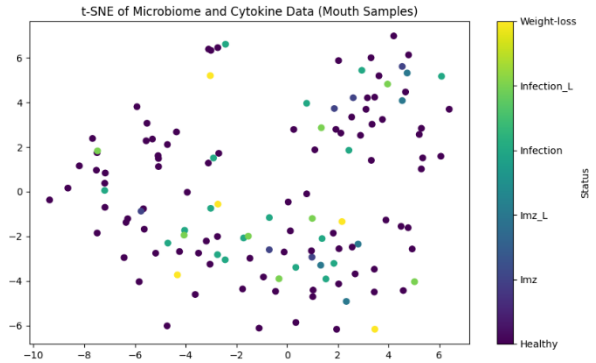
4.10 Dimensionality reduction and visualization

Principal Component Analysis (PCA) and t-distributed Stochastic Neighbor Embedding (t-SNE) were applied on combined microbial-cytokine features to visualize clustering by SampleType and health state (CL4).

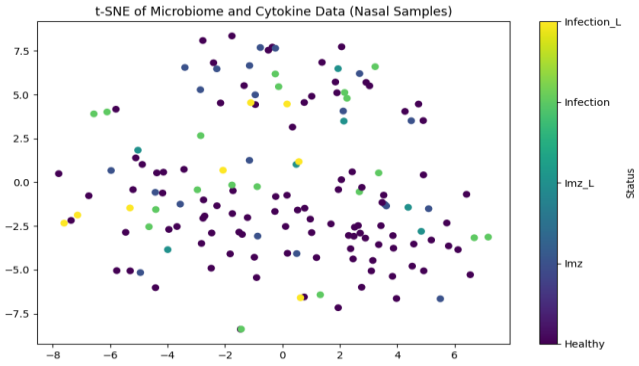
t-SNE of combined data, colored by SampleType and Health status(CL4)).



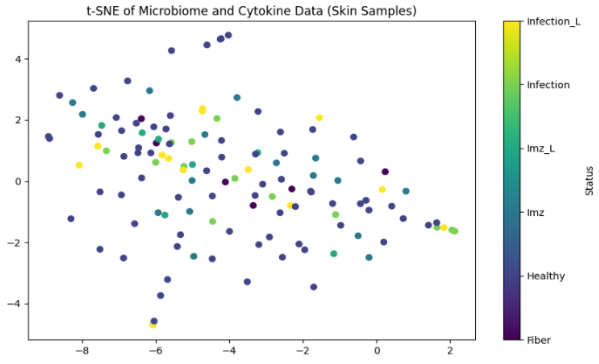
Stool



Mouth



Nasal



Skin

Figure 13

4.11 Temporal dynamics

Longitudinal samples were analyzed by grouping Shannon diversity and cytokine averages across collection years. Time-series trends were visualized using seaborn line plots, stratified by health state. This allowed detection of diversity erosion post-infection and partial recovery phases, suggesting therapeutic windows for intervention.

4.12 Reproducibility and implementation

All analyses were implemented in Python 3.9 using the following libraries: scikit-bio, scikit-learn, imbalanced-learn, seaborn, matplotlib, networkx, statsmodels, and SciPy. Logging utilities ensured full reproducibility with timestamps. Scripts were containerized in Kaggle runtime environments, with Kraken2/Bracken installed manually via shell scripts. Random seeds (42) were fixed for reproducibility.

5. Conclusion

In this landmark analysis, we unveil the first integrated atlas of human microbial-immune crosstalk across four anatomical niches, positioning LEPTIN as a ubiquitous cytokine orchestrator and the nasal microbiome as an exquisitely sensitive sentinel for systemic perturbations. Transcending mere associations, our multi-omics integration yields high-fidelity, site-specific biomarkers such as nasal LEPTIN-Labilibaculum pairings for early infection detection that empower AI-augmented classifiers with up to 98% accuracy, surpassing unimodal approaches. This blueprint for ecosystem medicine charts transformative pathways: non-invasive swab-based diagnostics, precision probiotics targeting pathobiont-cytokine hubs (e.g., oral TNFA-Fusobacterium), and temporal interventions to avert diversity erosion post-perturbation. Ultimately, by decoding the symbiotic lexicon of microbes and immunity, we illuminate a future of preventive, personalized care mitigating disease onset before clinical manifestation and reshaping global health paradigms for equitable, resilient outcomes.

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