

P3.133: Lung Bugs and the Metabolic Chaos in Non-Small Cell Lung Carcinoma

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INTRODUCTION

- Non-small cell lung carcinoma (NSCLC) remains the top cause of cancer-related mortality globally.
- The Microbiome Challenge:** While the lung microbiome is linked to NSCLC biology, distinguishing true tumour-associated signals from background airway microbiota or upper-airway contamination difficult.
- Indian Context:** India faces high ambient air pollution; since the lung microbiome is exposed to inhaled particles, Indian NSCLC patients may harbour unique microbial signatures compared to Western cohorts.

Objective

To determine if the NSCLC tumour microbiome represents a structured, functionally coherent community or merely a passive collection of microbes.

METHODS

Design:

- Cohort:** Prospective collection of surgically resected lung tissue from **13 Indian NSCLC patients** following a **Paired design** using Tumour (T) and matched Normal-Adjacent Tissue (NAT) from each patient, plus **6 Healthy-Donor controls** (HC) to isolate tumour-specific signals.
- Sequencing:** Shotgun metagenomic sequencing (Illumina NovaSeq 6000) following host DNA depletion.
- Filtering Cascade:** A rigorous **three-step strategy** removed taxa enriched in healthy controls, shared disease-associated background signals, and low-prevalence features (Fig. 1).
- Analysis:** Integrated paired differential abundance (DESeq2, ANCOM-BC2), phylogenetic grouping (Woltka), and functional profiling (HUMAN3).

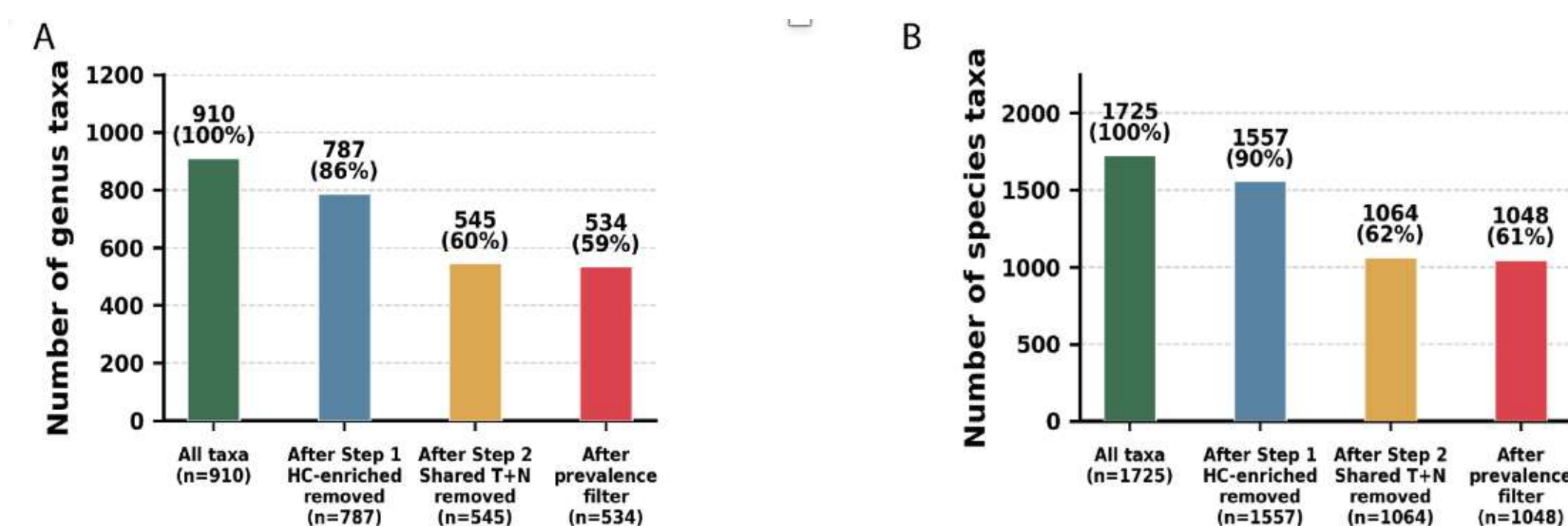


Figure 1. (A) Three-step filtering cascade applied at the genus level, showing the number of taxa retained after removal of healthy control-enriched taxa, shared tumour-plus-normal disease-associated taxa, and low-prevalence features. (B) Three-step filtering cascade applied at the species level, showing the number of taxa retained after the same filtering steps.

RESULTS

1. Paired Differential Abundance

- We Identified **17 tumour-enriched genera** and **5 normal-enriched genera** using DESeq2. Notable tumour-enriched taxa include *Nitrosocosmicus*, *Bacillus_A*, *Alloprevotella*, and *Moraxella* (Fig. 2).
- Overall alpha diversity** did not significantly differ between T and NAT, but the **compositional profile** did. The Aitchison distance comparisons between T and NAT showed that samples from the same patient were more similar to each other than samples from different patients (Figure 3).
- The agreement between ordination loadings and effect sizes ($r = 0.991$) confirms these signals are biologically robust and not methodological artefacts (Fig.4)

2. Phylogenetic Clades & Network Density

- Tumour-associated taxa grouped into **4 coordinated phylogenetic clades**: C6 (Bacteroidales), C11 (Bacillales), C20 (Cyanobacteriales), and C36 (Burkholderiaceae)
- Consistency:** Clade C11 was enriched in the tumour tissue of 12 out of 13 patient pairs (92.3%).
- Network Structure:** Tumour tissue showed a **denser co-occurrence network** (2,382 connections) compared to normal tissue (1,769), with tumour-associated taxa occupying central positions, indicating tumor microbe associations.

3. Functional Pathway Shifts (Fig. 5)

- Tumour-Associated Functions:** Clades were linked to **oxidative phosphorylation**, **reactive oxygen species (ROS) production**, **DNA recombination**, and **hypoxia response**.
- Normal-Associated Functions:** Linked to metabolic maintenance, detoxification (Cytochrome-P450), and bacterial housekeeping.

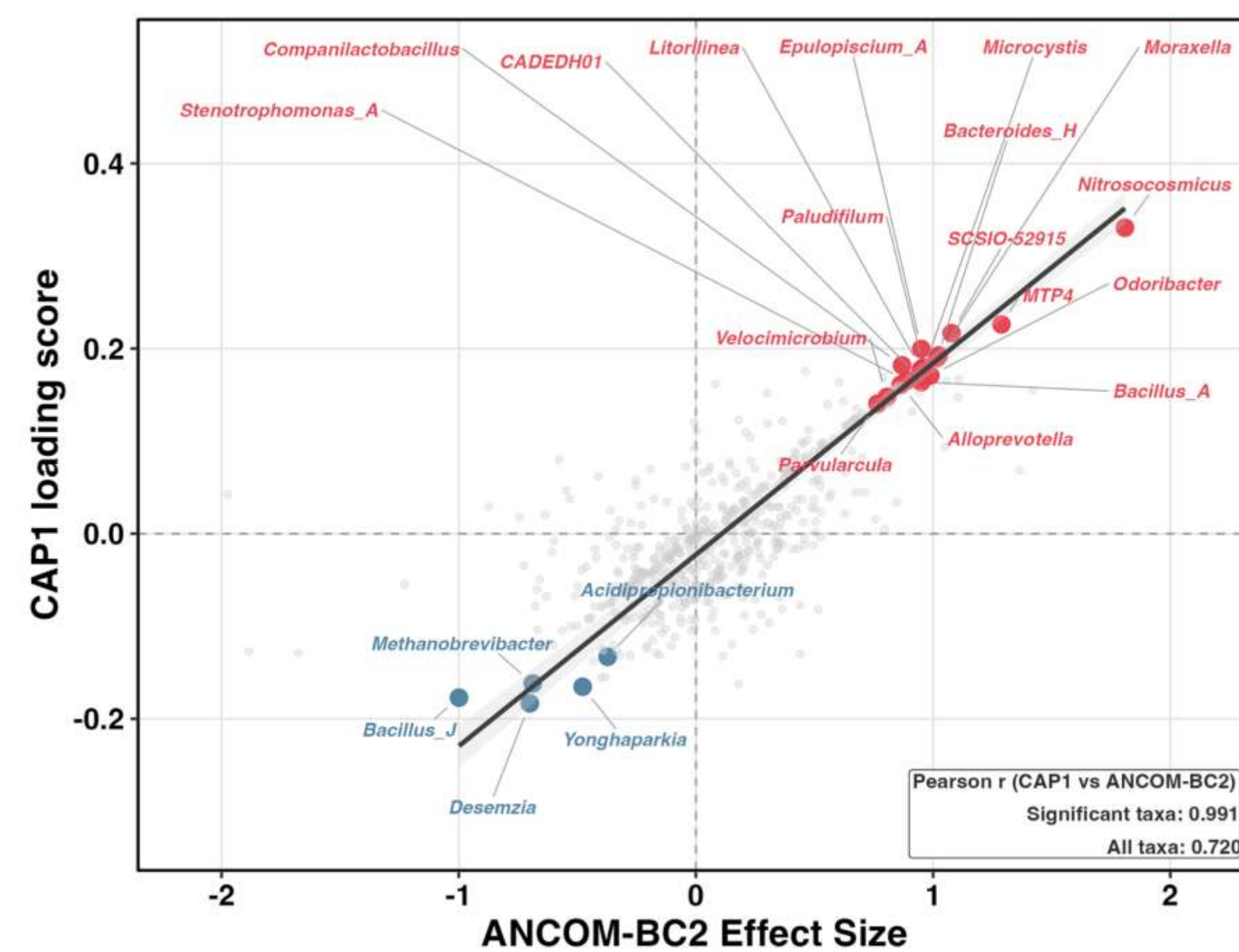


Fig.4: Relationship between CAP1 loadings from patient-partialled db-RDA and ANCOM-BC2 log2 fold changes across 534 genera. Red points denote DESeq2 tumour-enriched genera and blue points denote normal-enriched genera. CAP1 loading and ANCOM-BC2 effect size showed a strong positive Pearson correlation among significant taxa ($r = 0.991$) and across all tested taxa ($r = 0.720$).

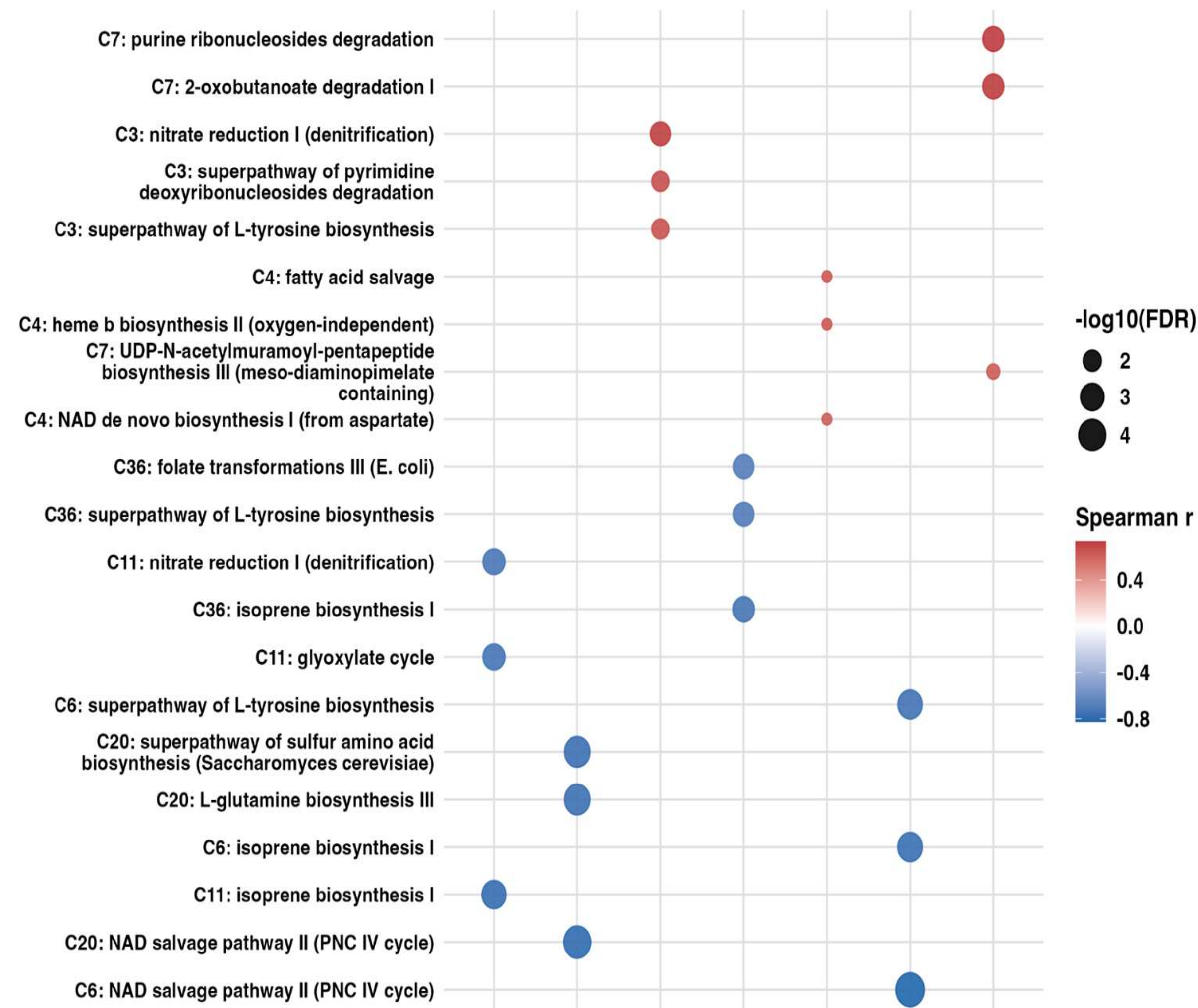


Fig.5: Bubble plot of representative concordant clade-pathway associations across selected phylogenetic clades. Each row denotes a pathway and each column denotes a clade. Dot size corresponds to $-\log_{10}(\text{FDR})$, and dot colour indicates the direction and magnitude of the clade-pathway Spearman correlation. Only pathways showing significant clade-level association and concordant tumour-versus-normal community-level direction are displayed.

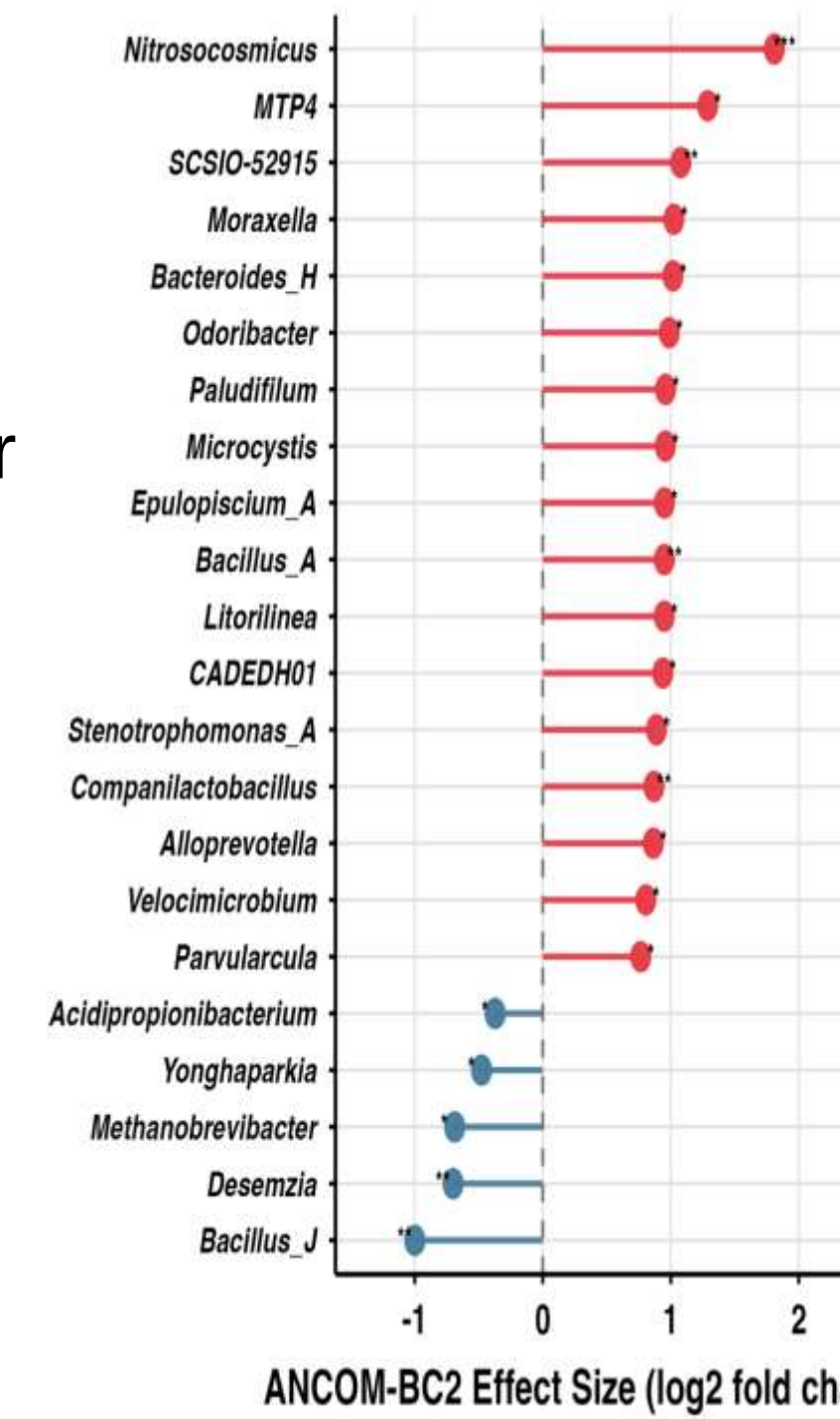


Fig. 2 : Genus enriched in Tumor and Normal Samples

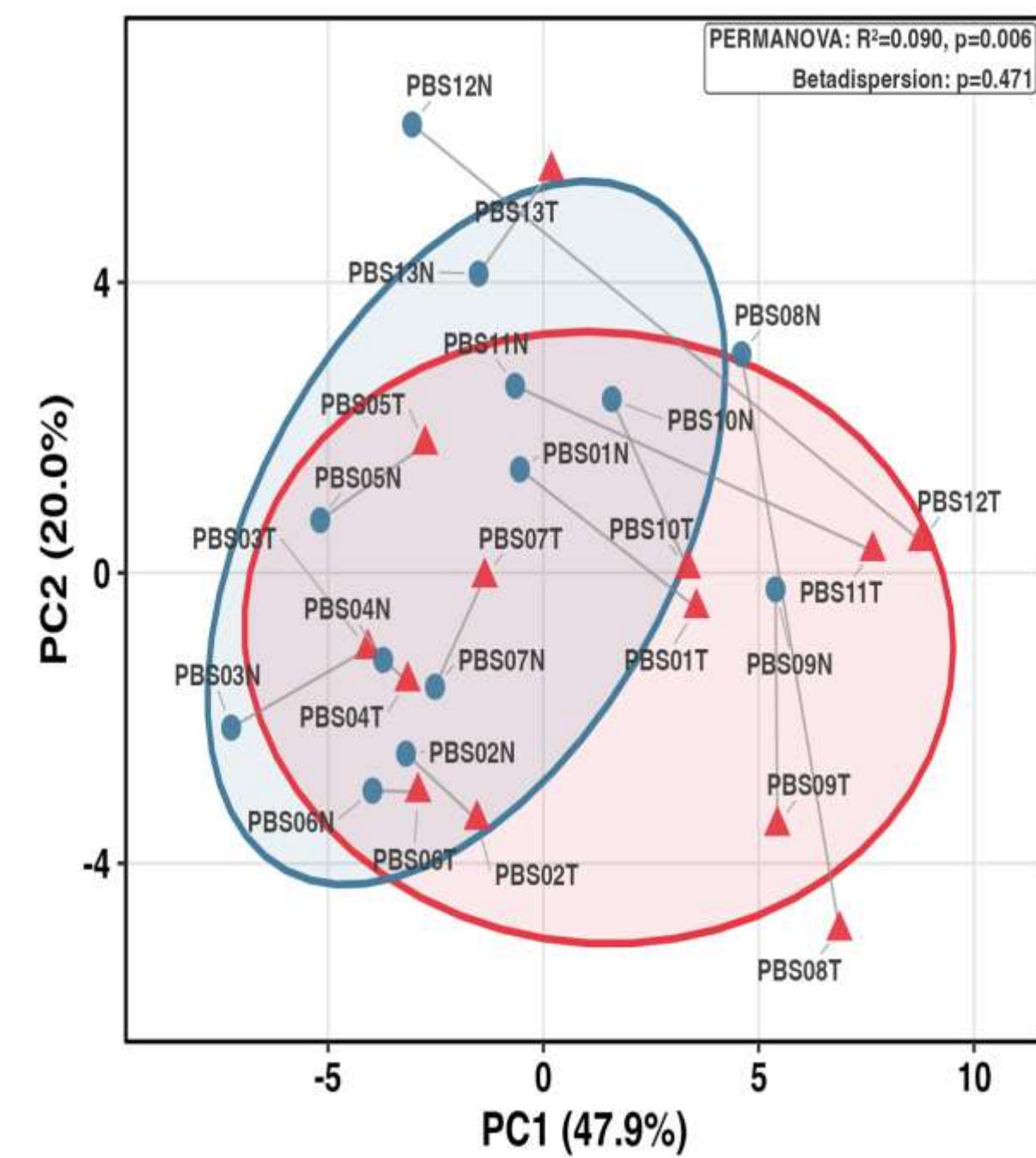


Fig. 3: Aitchinson distance comparison shows similar compositions between the tumor - normal adjacent tumor.

DISCUSSION AND CONCLUSION

Actionable Take Away Message

- Structured Community:** Intratumoral microbes in NSCLC are not incidental; they form **structured, functionally organised communities** rather than passive colonisers.
- Environmental Impact:** Detection of taxa like *Nitrosocosmicus* (ammonia-oxidizing) and *Cyanobacteriales* (clade C20) likely reflects high environmental nitrogen/pollution exposure (in India), potentially contributing to genotoxicity.
- Metabolic Shift:** The overall microbiome shifts from "housekeeping" functions in normal tissue to pathways supporting tumour biology (ROS, Hypoxia).

Future Direction for Research

- Validate findings on larger cohort. Additionally using spatial methods find the localisation of microbes within the tumour microenvironment
- Understand microbiome mediated immune responses in NSCLC.

REFERENCES & ACKNOWLEDGEMENTS

- Key references : Dickson & Huffnagle (2015), Peters et al. (2022), and Li et al. (2024)
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