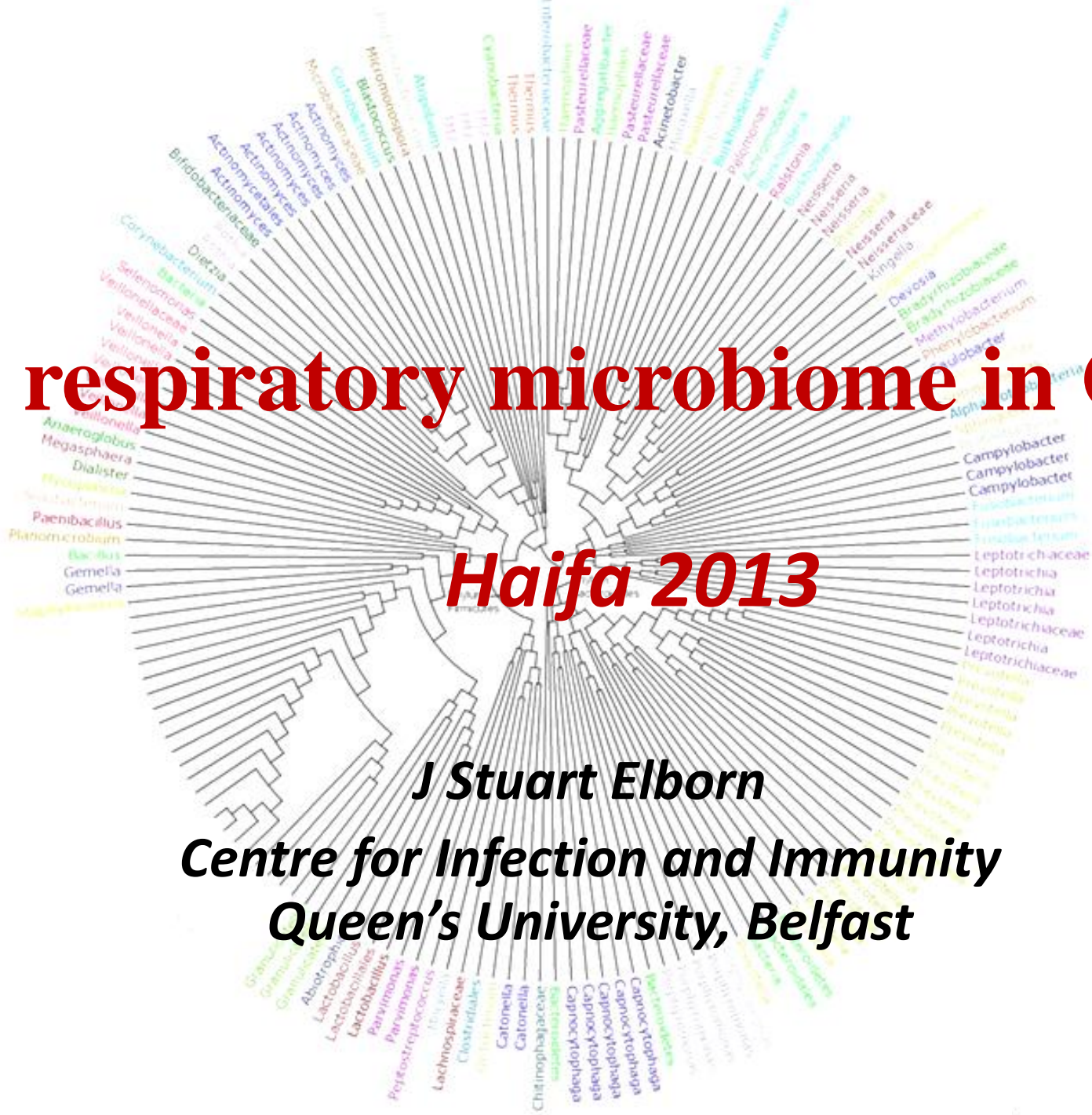


# The respiratory microbiome in CF

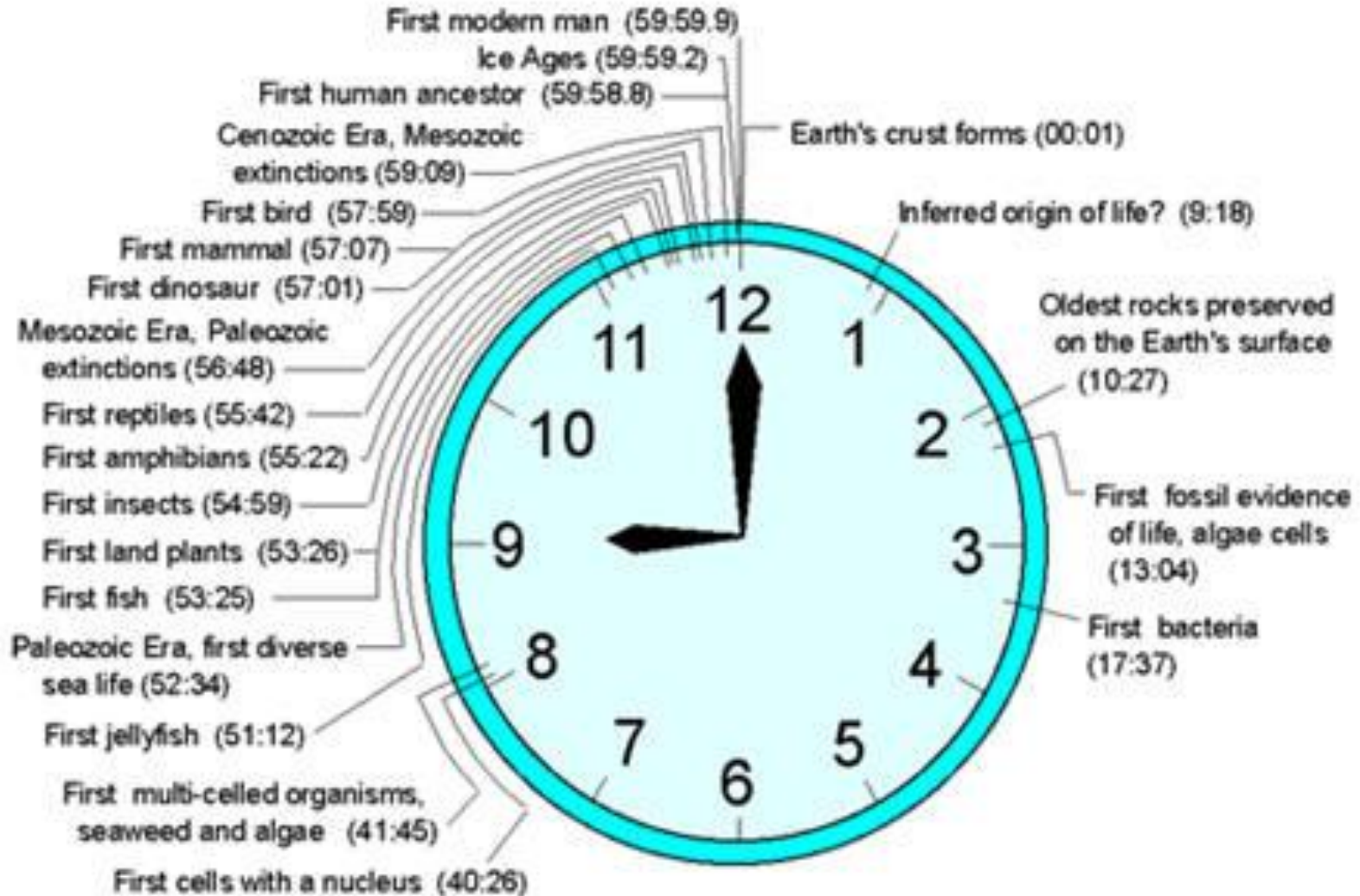
*Haifa 2013*

*J Stuart Elborn*

*Centre for Infection and Immunity  
Queen's University, Belfast*

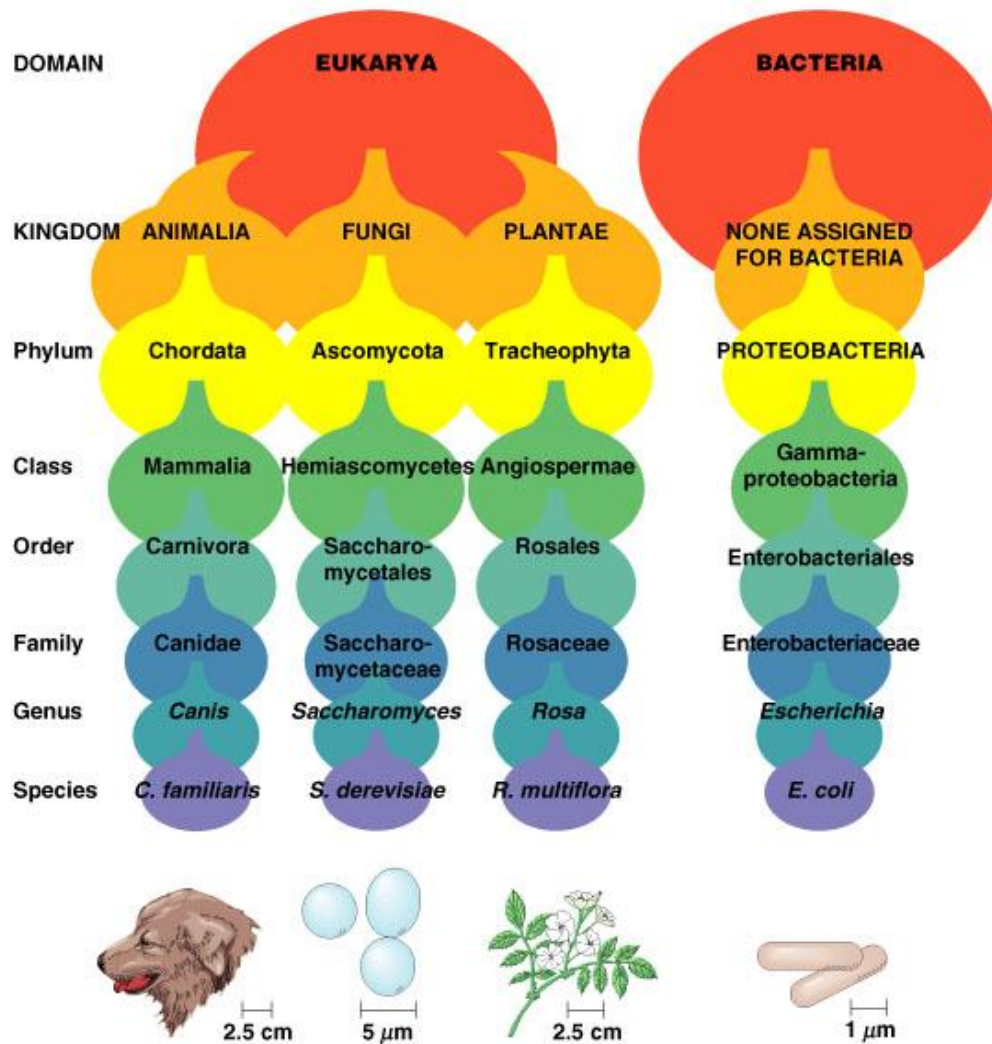


# Evolution of life

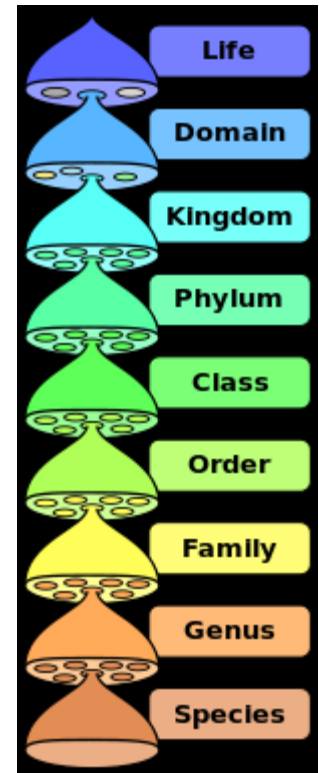


4.6 billion years in one hour

# Classification of Bacteria

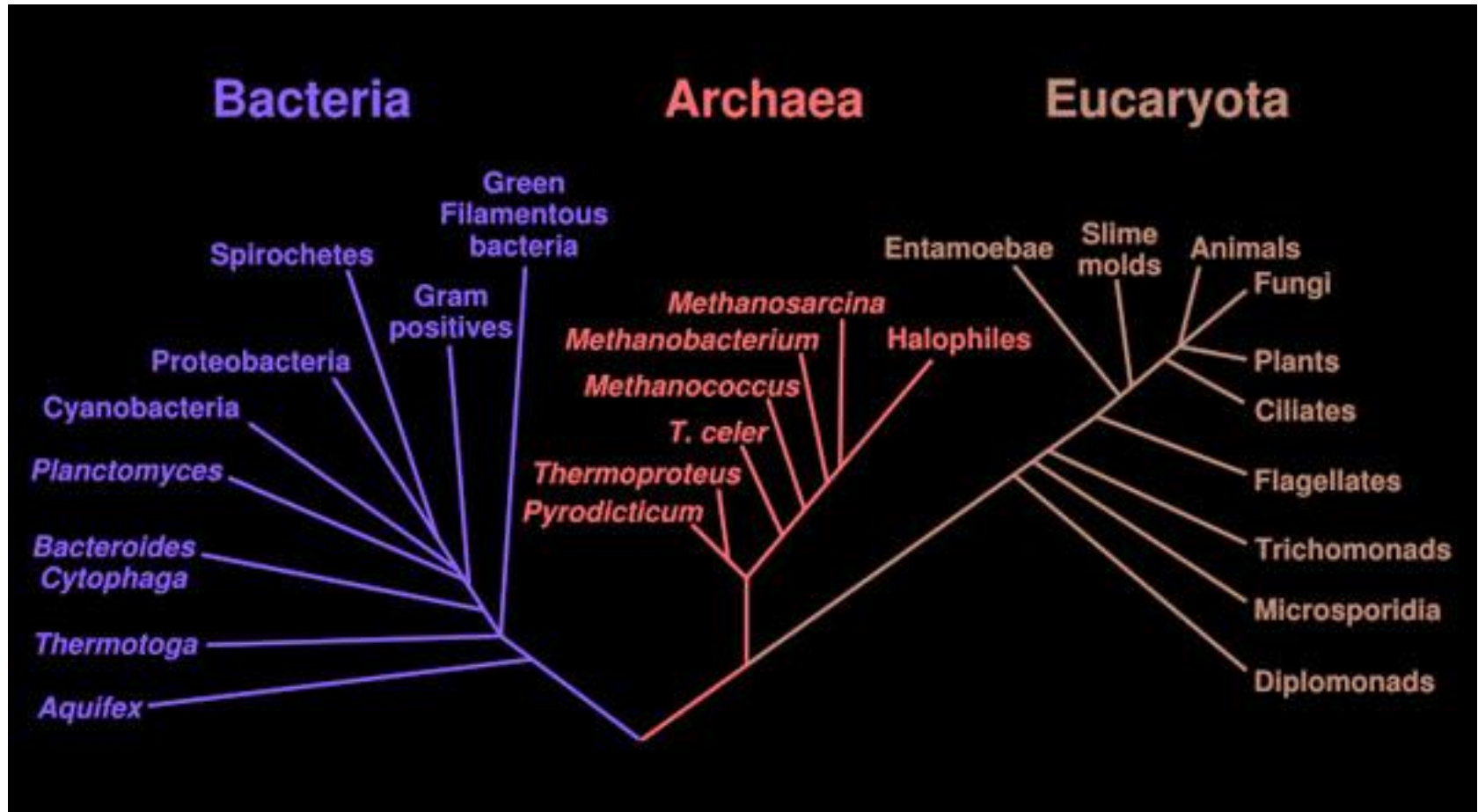


Copyright © 2004 Pearson Education, Inc., publishing as Benjamin Cummings.



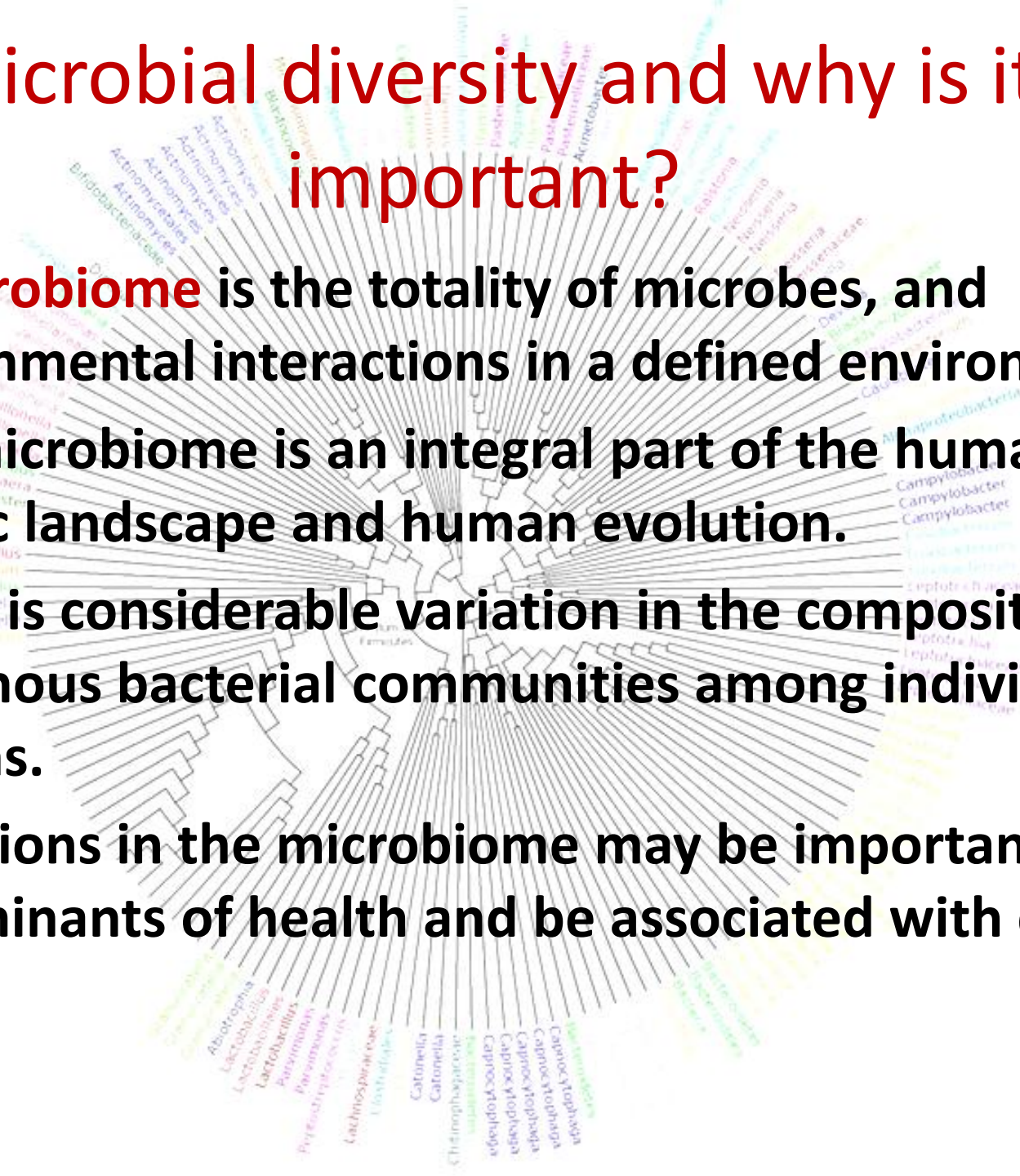


# Classification of life

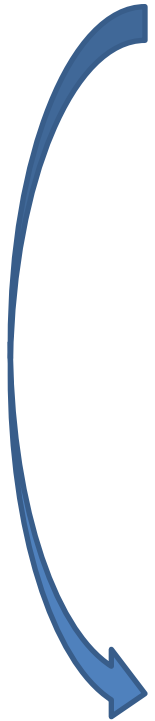


# Microbial diversity and why is it important?

- A **microbiome** is the totality of microbes, and environmental interactions in a defined environment.
- The microbiome is an integral part of the human genetic landscape and human evolution.
- There is considerable variation in the composition of indigenous bacterial communities among individual humans.
- Variations in the microbiome may be important determinants of health and be associated with disease.



# Definitions

- 
- Microbiome:  
the totality of microbes & their genomes in a defined environment (the gene complement of a microbial community)
  - Microbial Community:  
collection of microbes existing in the same place at the same time
  - Microbiota:  
the microbes that inhabit a defined niche

# Measuring Biodiversity

- 🧬 In simple terms the measure of biodiversity is the number of counted species/taxa in a sample → “**species richness**”.
- 🧬 Another concept of species diversity is “**abundance**”:

	<i>Community 1</i>	<i>Community 2</i>
<b>Species 1</b>	<b>99</b>	<b>50</b>
<b>Species 2</b>	<b>1</b>	<b>50</b>

- 🧬 Where the “heterogeneity” is higher in a community where there are more species and when the species are more abundant.

# Diversity Indices

- 🧬 Are a mathematical measurement of species diversity in a community.
- 🧬 This can reveal an important information regarding rarity and/or commonness of species in a specific community.
- 🧬 The diversity within an ecological community that incorporates both species richness (i.e. the number of species in a community) and the evenness of species abundances.
- 🧬 Examples of diversity metrics commonly used are the Shannon-Wiener Index and Simpson's Index.



# The Human Microbiome

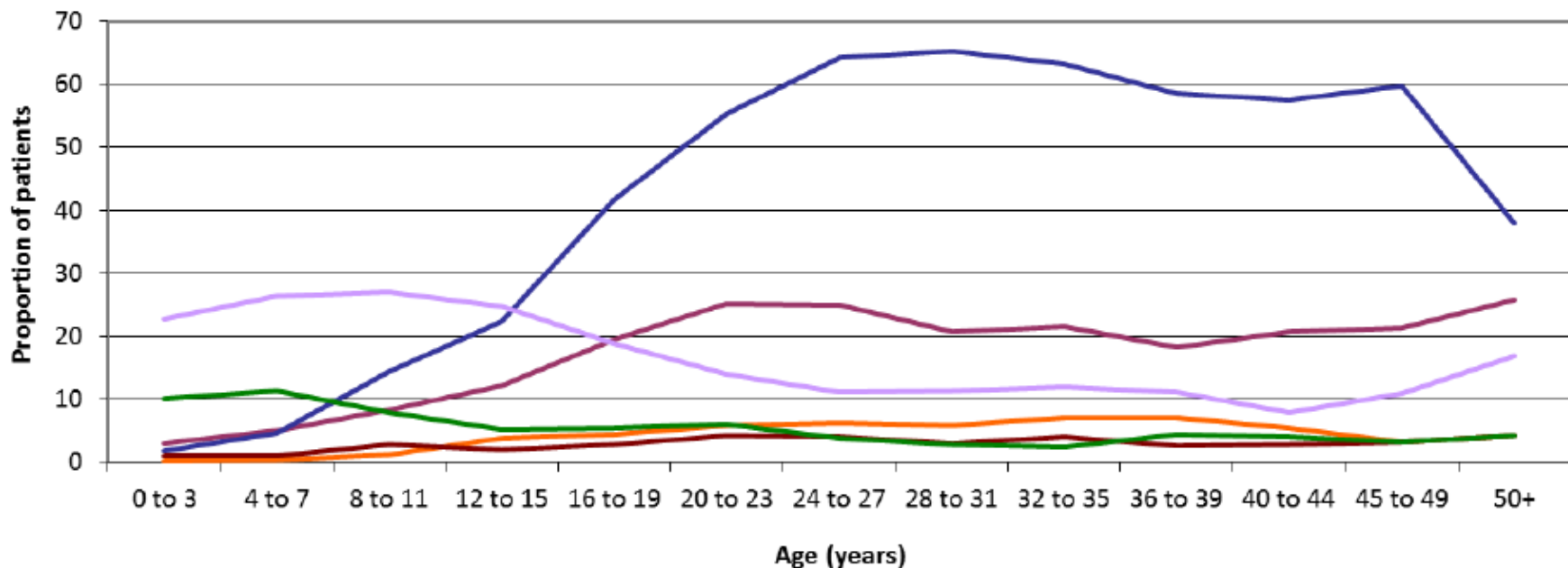


# Microbiota and the airways

- Estimated that the human **microbiota** is composed of  $\sim 10^{14}$  bacterial cells, outnumbering human cells 10:1.
- The microbiota and human cells form a complex ecosystem that as a whole, interactively performs various biological processes.
- Only 40% of bacteria can be cultured by conventional methods.
- Airways are not sterile and under constant challenge from inhaled bacteria.
- No permanently resident flora?
- In airway diseases, such as CF, BE and COPD the local airway defences are compromised leading to new infections and exacerbations by opportunistic micro-organisms.

# Airway infection in CF

1.17 Lung infections in 2011



Chronic *S.aureus*; n=1312 (15.7%)

Chronic *P.aeruginosa*; n=3058 (36.5%)

Intermittent *P.aeruginosa*; n=1547 (18.4%)

*B.cepacia*; n=314 (3.8%)

MRSA; n=221 (2.6%)

*H.influenzae*; n=523 (6.2%)

# You only find what you look for....

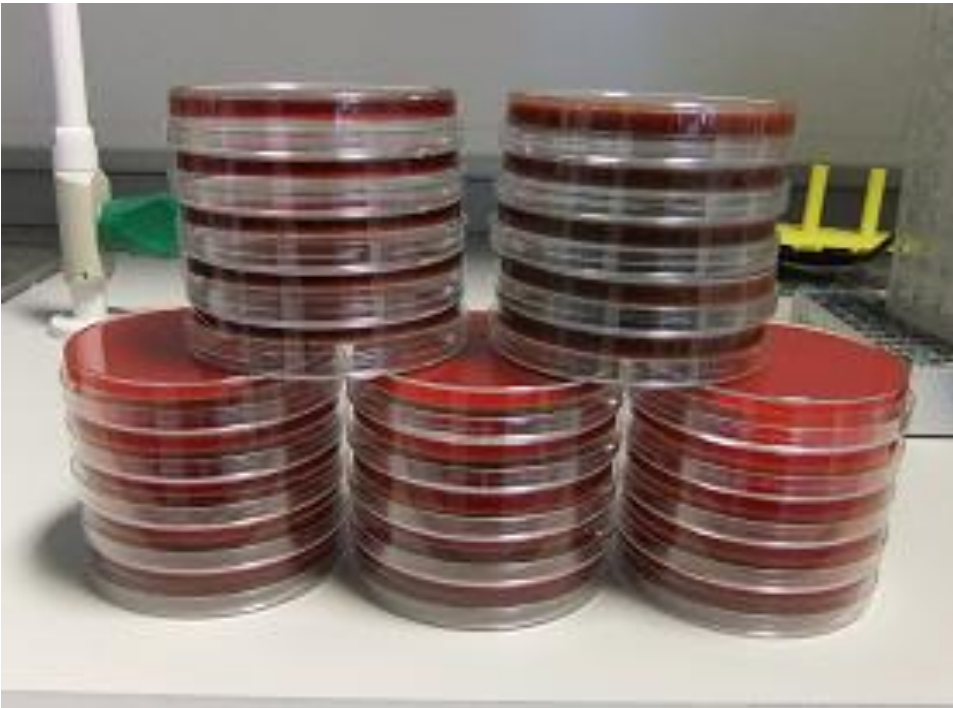
- Aerobic culture at RT/37°C
- Specific media
- Biochemical ID
- Maldi-TOF
- Molecular
  - specific PCR
  - Broad range PCR and sequencing of 16S ribosomal RNA genes
- Ecological approaches  
(Metagenomics)
  - T-RFLP
  - 2<sup>nd</sup> generation sequencing





# Sputum Processing

## *media and conditions*



Aerobes

- $O_2$
- $37^{\circ}C$

Microaerophiles

- 5%  $CO_2$
- $37^{\circ}C$

Anaerobes

- Anaerobic gas
- $37^{\circ}C$

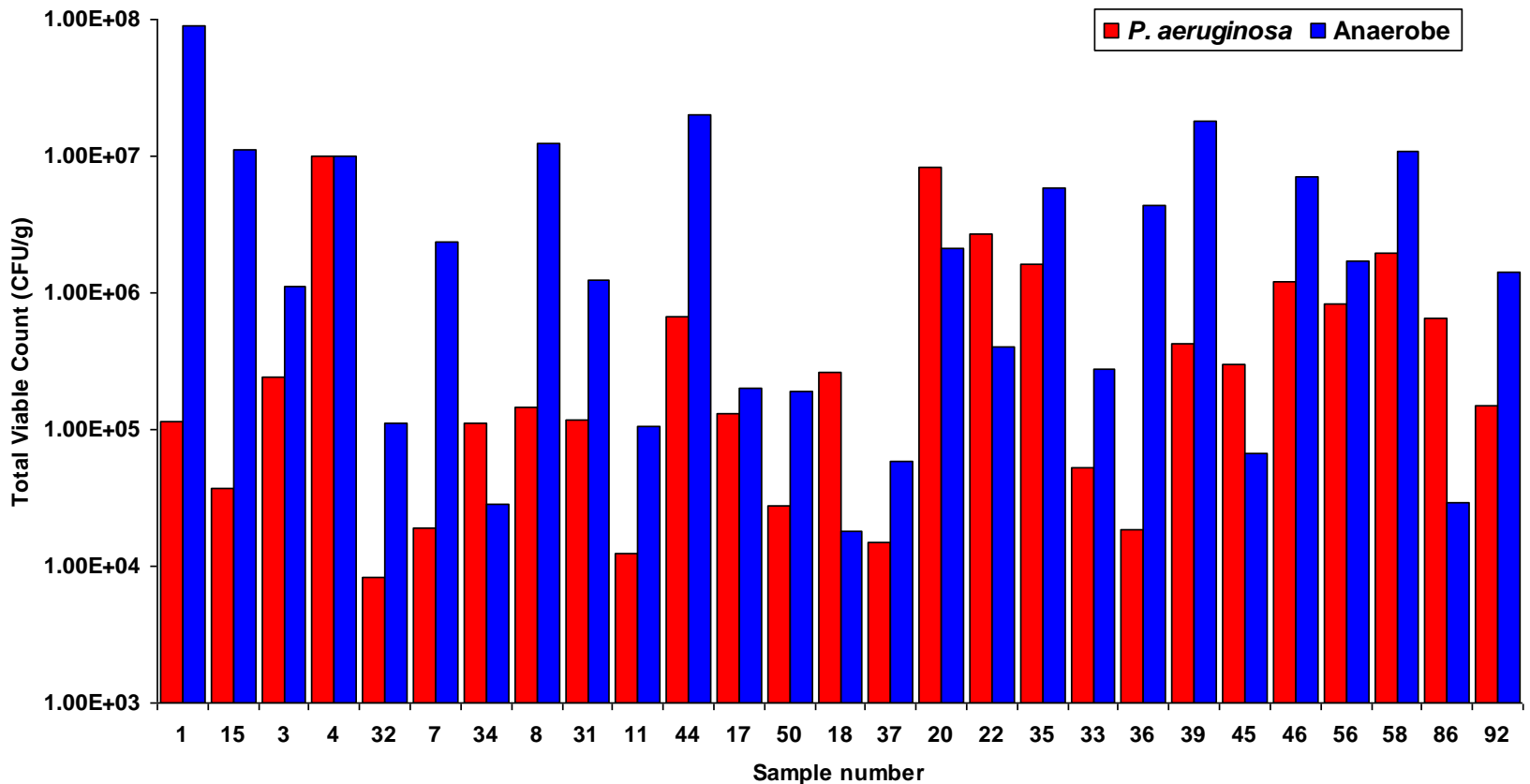
# The “culture” microbiota



# Comparison of Viable Counts

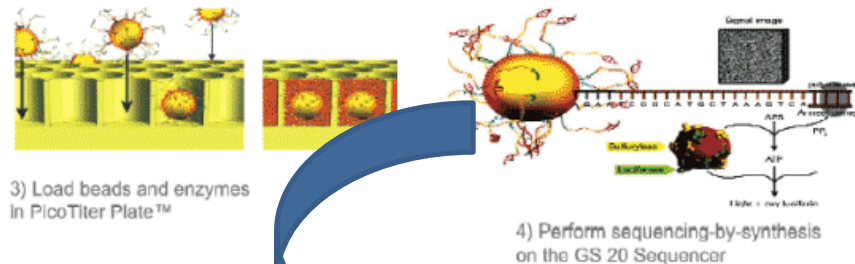
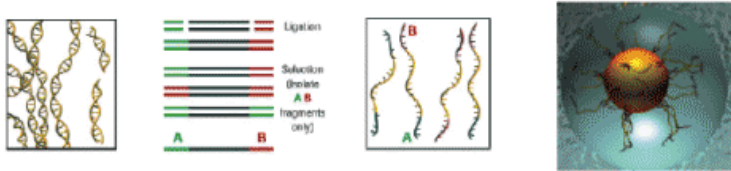
## *Extended culture*

- 14 different genera (78 isolates)
- *Prevotella*, *Streptococci*, *Veillonella*, *Actinomyces* most prevalent



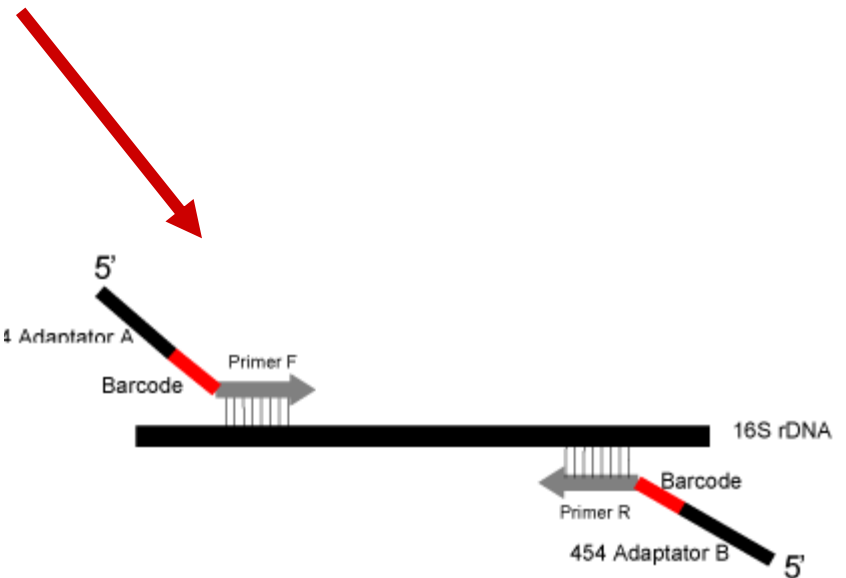
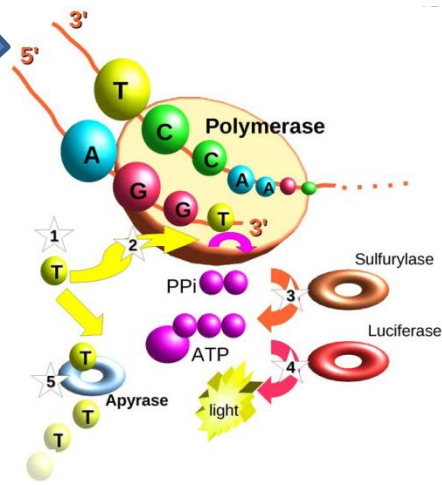
# 454 Pyrosequencing

Figure 1. Overview of the 454 sequencing system



[www.roche-applied-science.com](http://www.roche-applied-science.com)

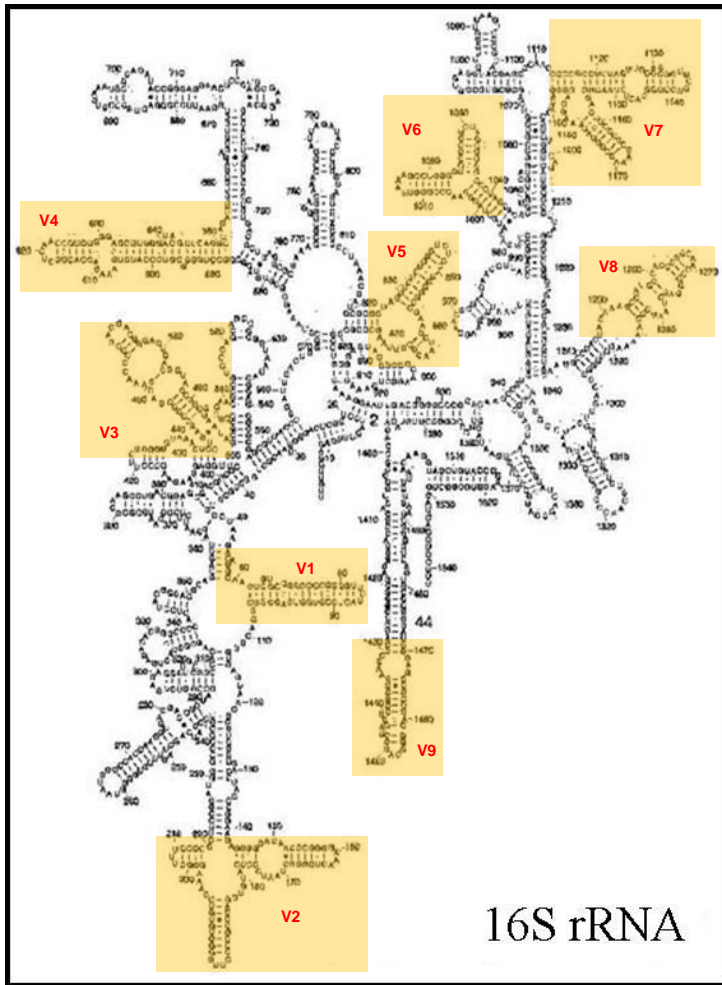
454 LIFE SCIENCES





# Molecular microbial community profiling

## – 16S rRNA gene sequencing



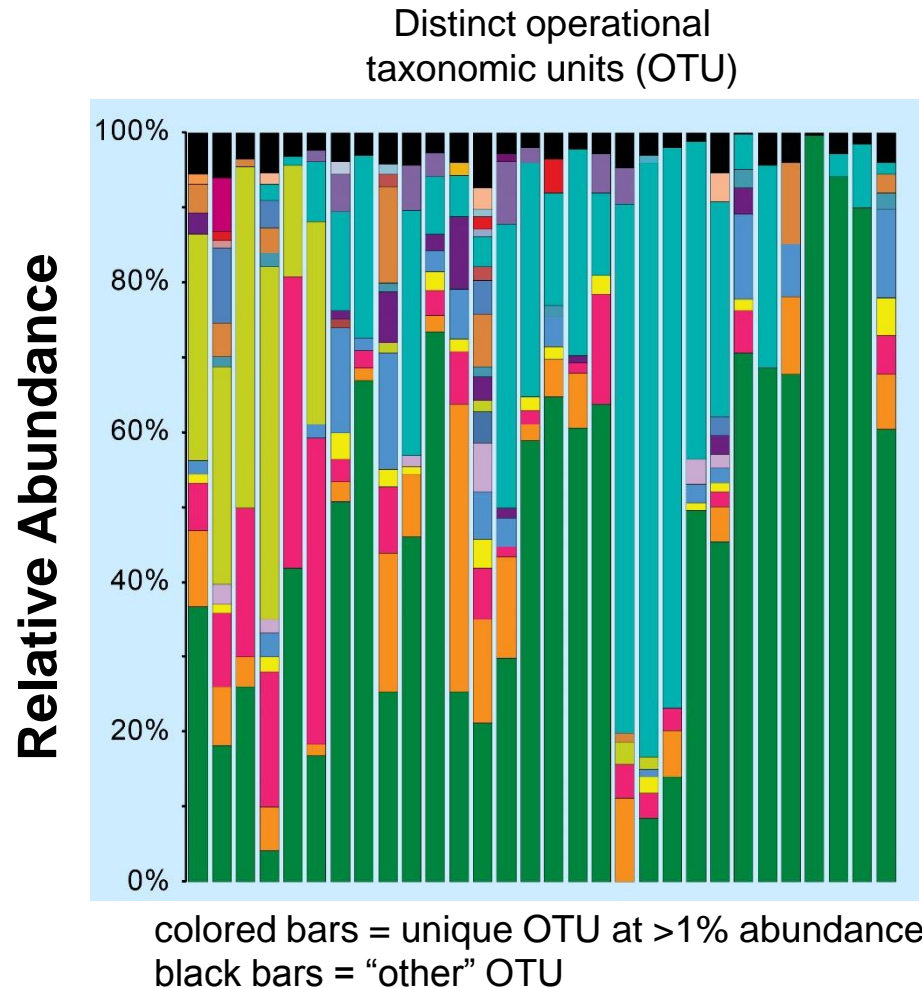
- 16S rRNA forms part of bacterial ribosomes
- Contains regions of highly conserved and highly variable sequence
- Variable sequence can be thought of as a molecular “fingerprint”
  - can be used to identify bacterial genera and species
- Large public databases available for comparison
  - RDP currently contains ~2 million 16S rRNA sequences
- Conserved regions can be targeted to amplify broad range of bacteria from clinical samples

Circumvents the need to culture – Not limited to targeted organisms

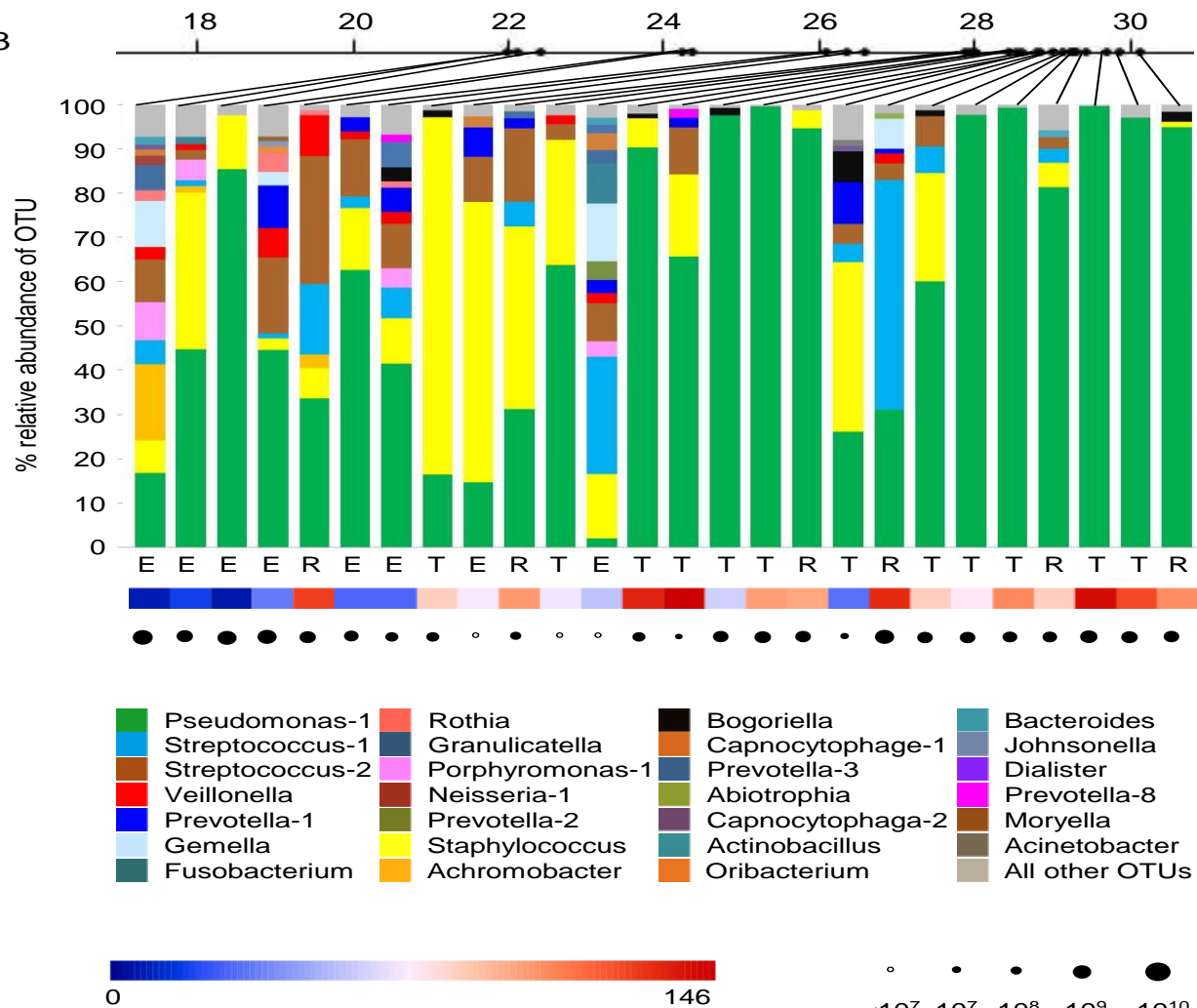


# Airway microbial diversity

30 sputum samples over >8 yrs from an adult with CF



# New approaches to managing airway infection



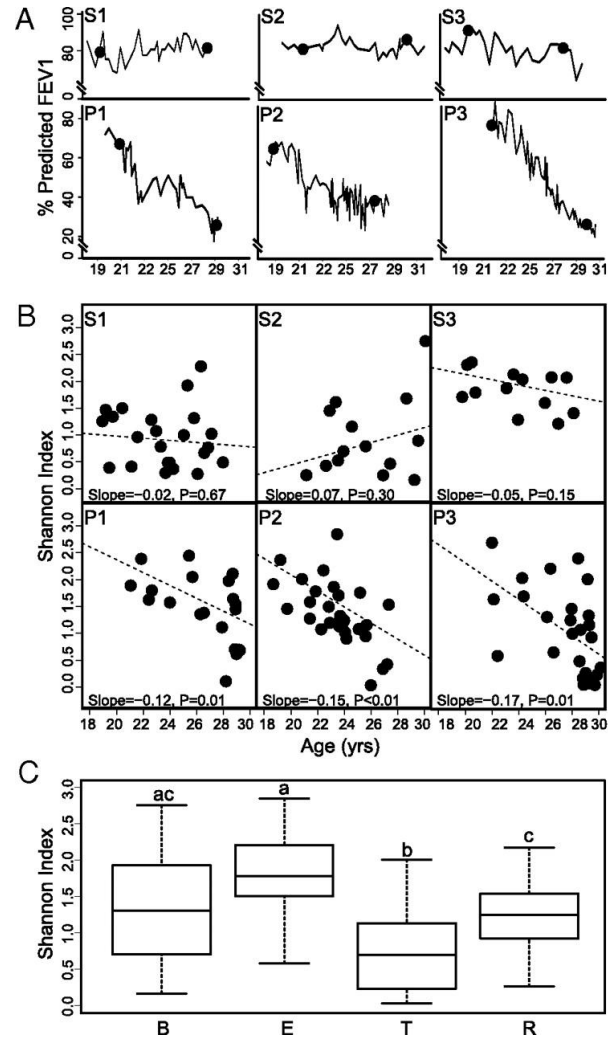
Decreasing diversity  
with age, disease  
severity, antibiotic  
exposure

Total bacterial density  
stays relatively stable

Is there a way to  
maintain 'healthier'  
communities?

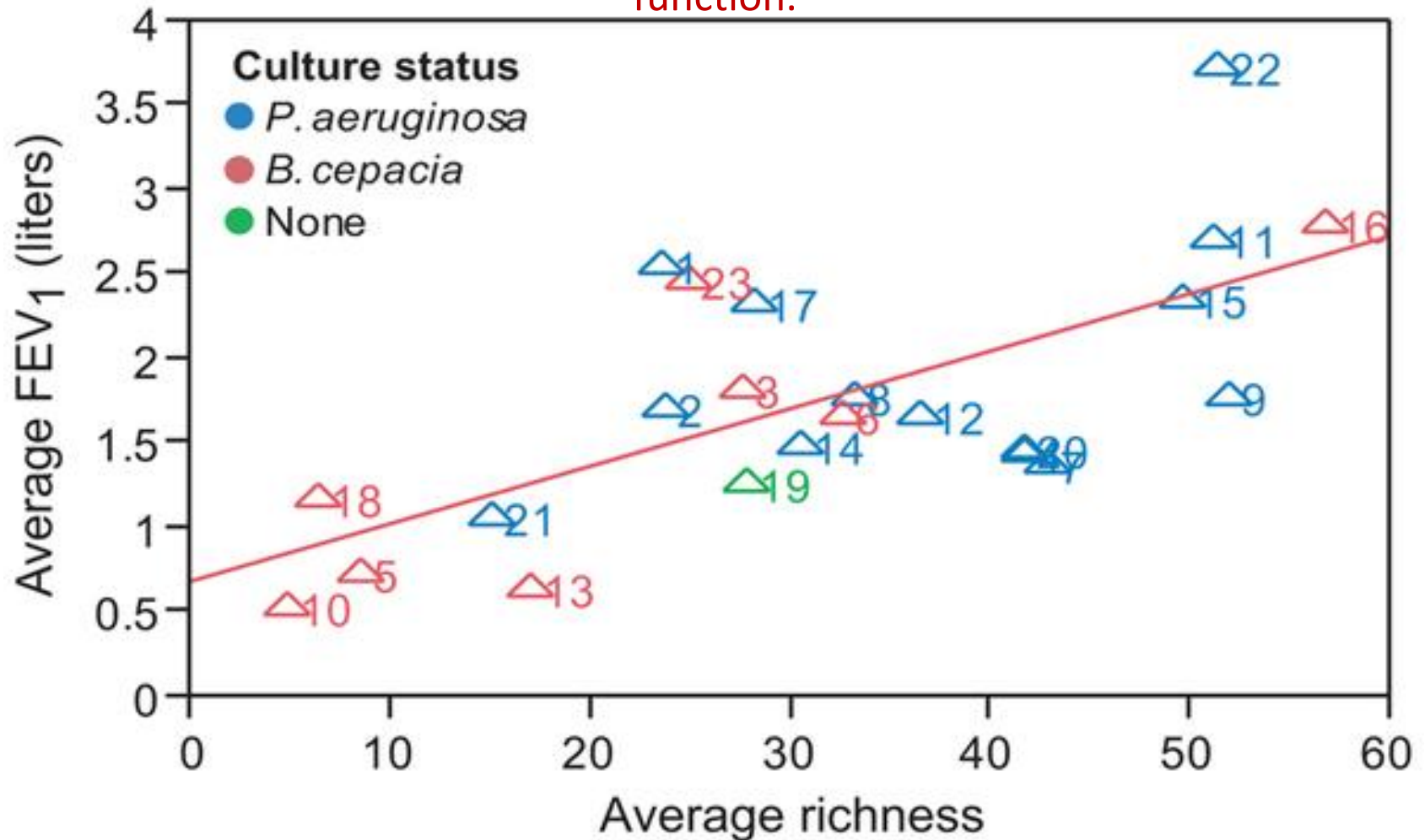


# Changes in lung function and community diversity.



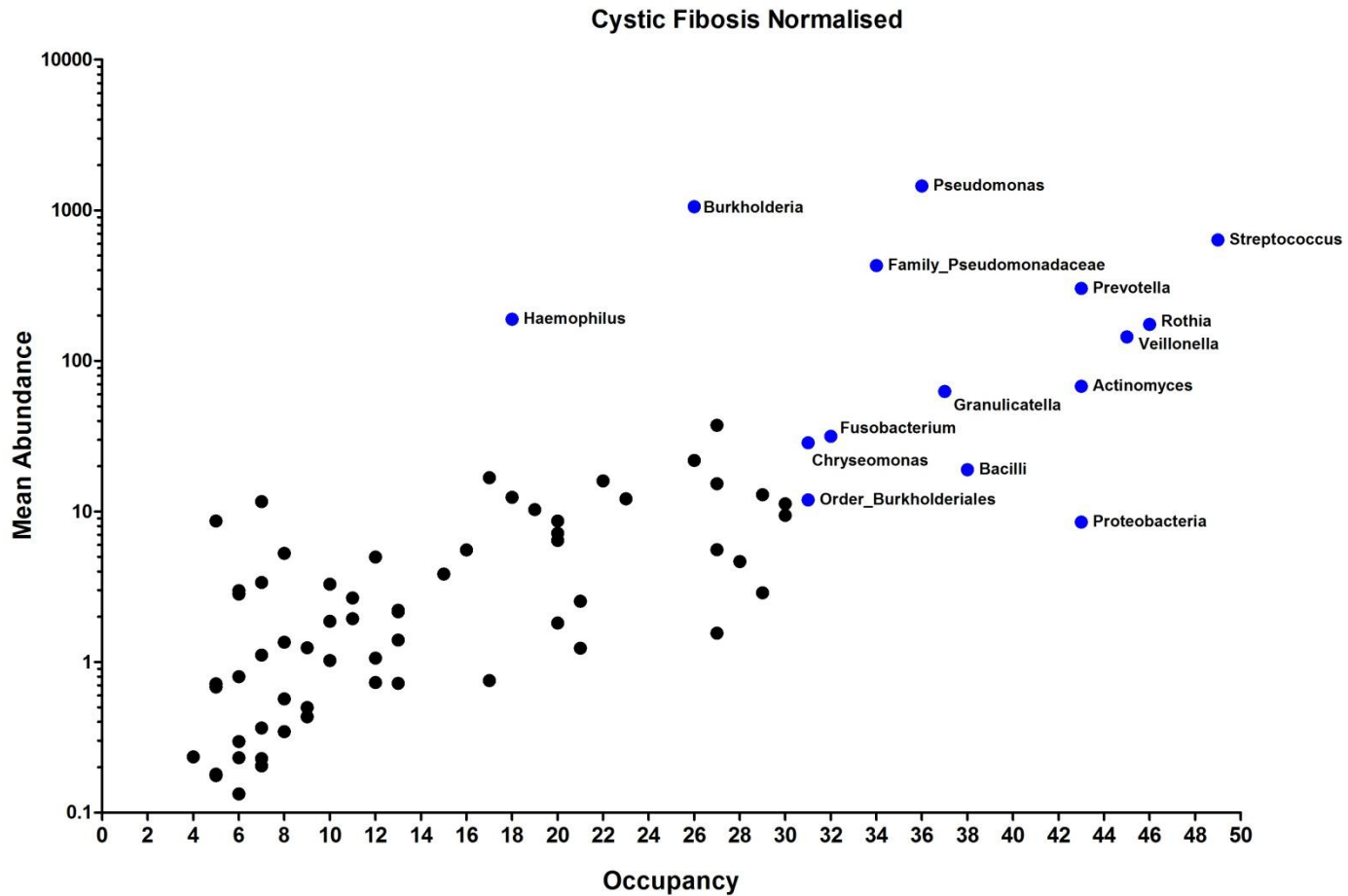
Zhao J et al. PNAS 2012;109:5809-5814

Low species richness in sputum samples is associated with decreased lung function.

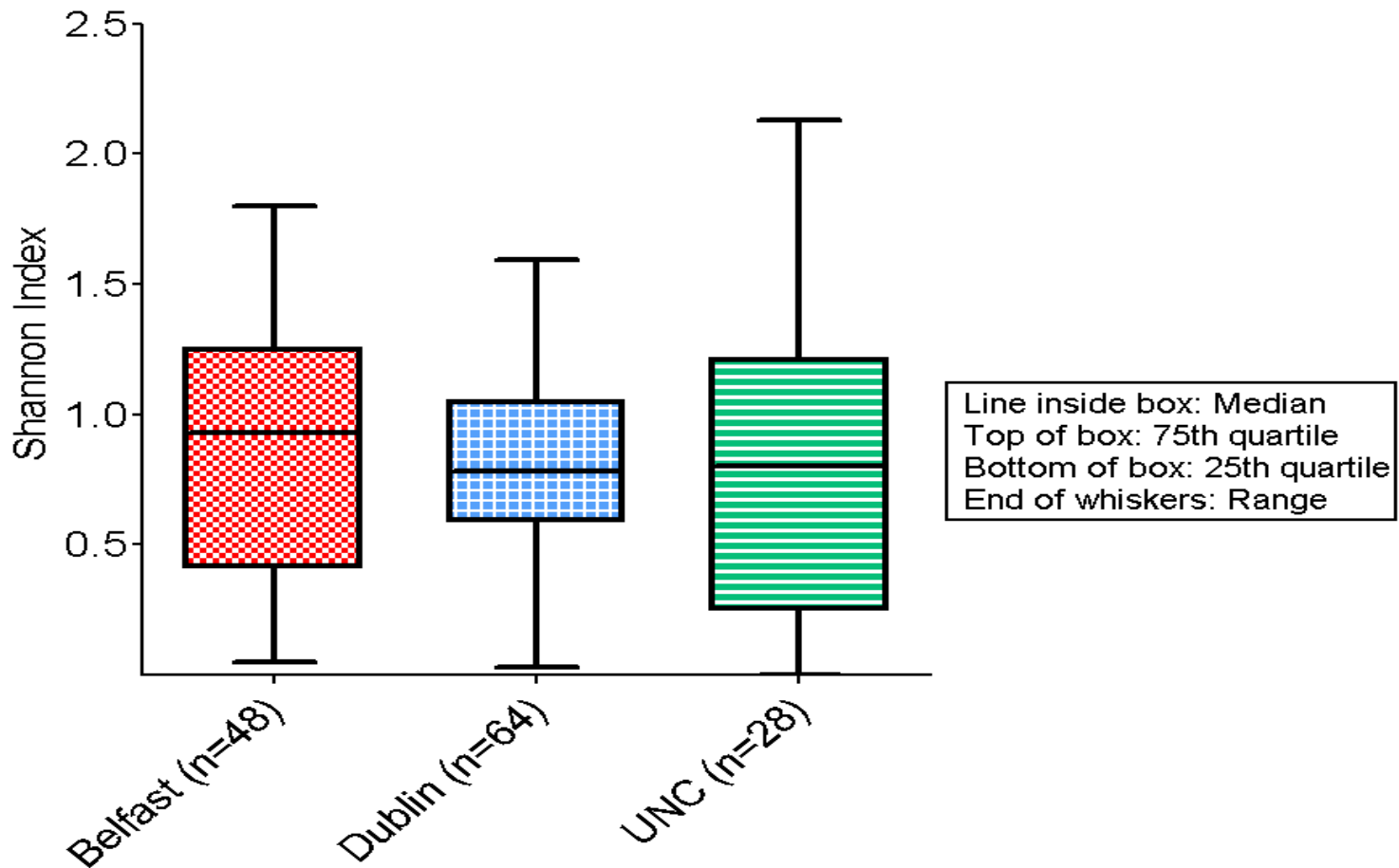


Fodor AA, Klem ER, Gilpin DF, Elborn JS, et al. (2012) The Adult Cystic Fibrosis Airway Microbiota Is Stable over Time and Infection Type, and Highly Resilient to Antibiotic Treatment of Exacerbations. PLoS ONE 7(9): e45001. doi:10.1371/journal.pone.0045001  
<http://www.plosone.org/article/info:doi/10.1371/journal.pone.0045001>

# Taxa Occupancy CF

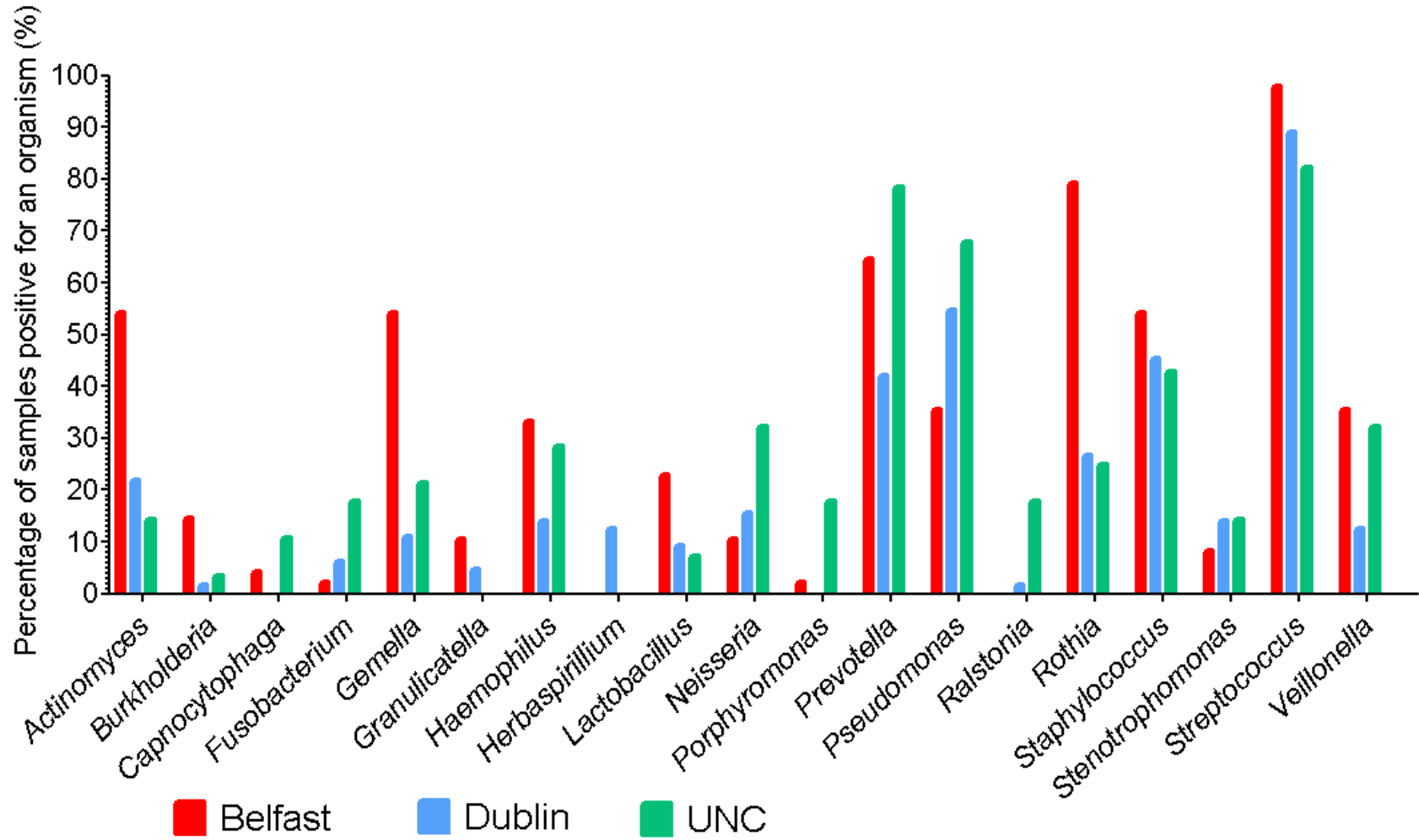


# Microbial diversity



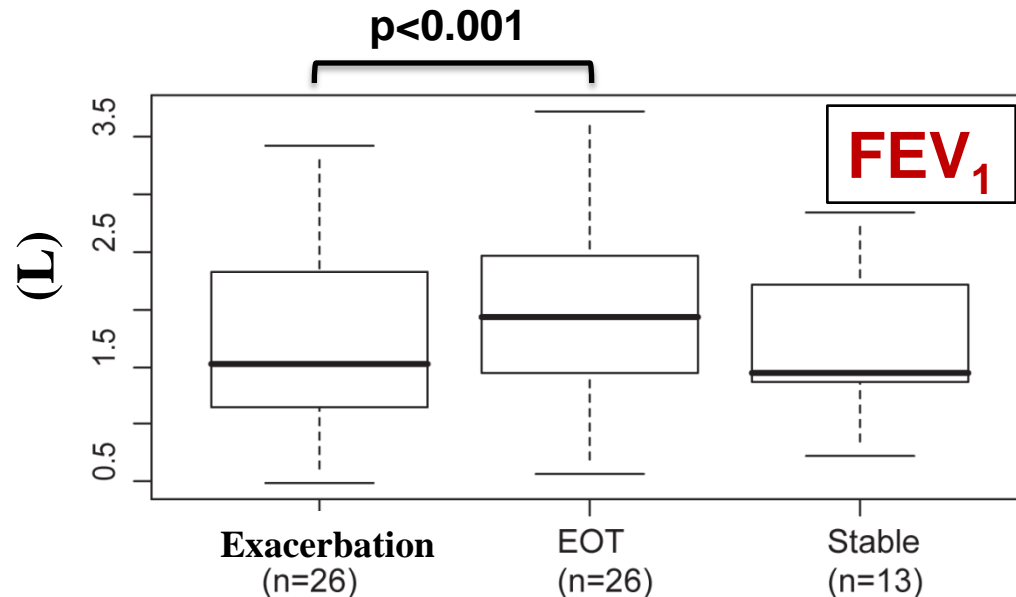
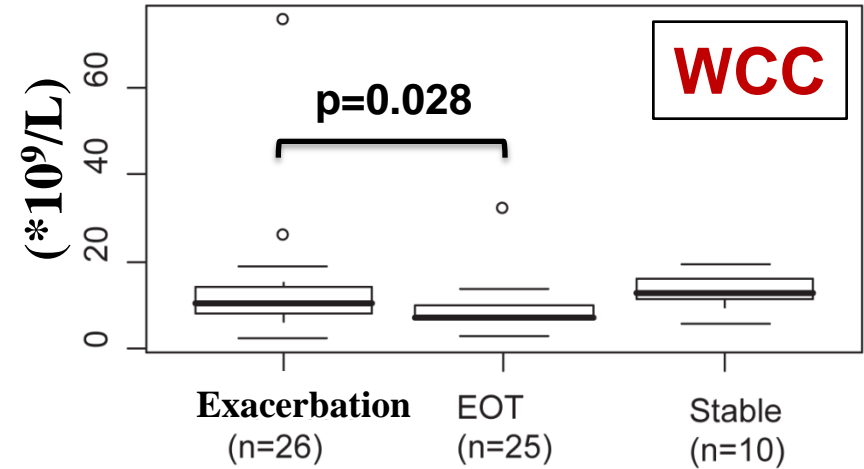
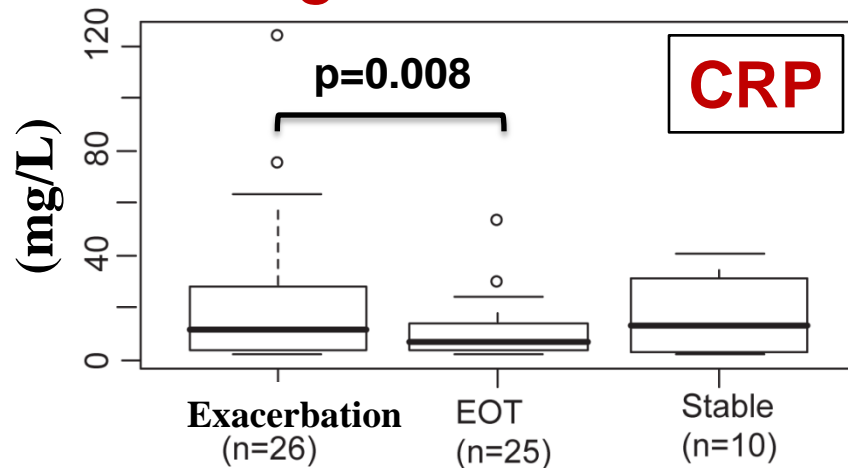


# Genera detected

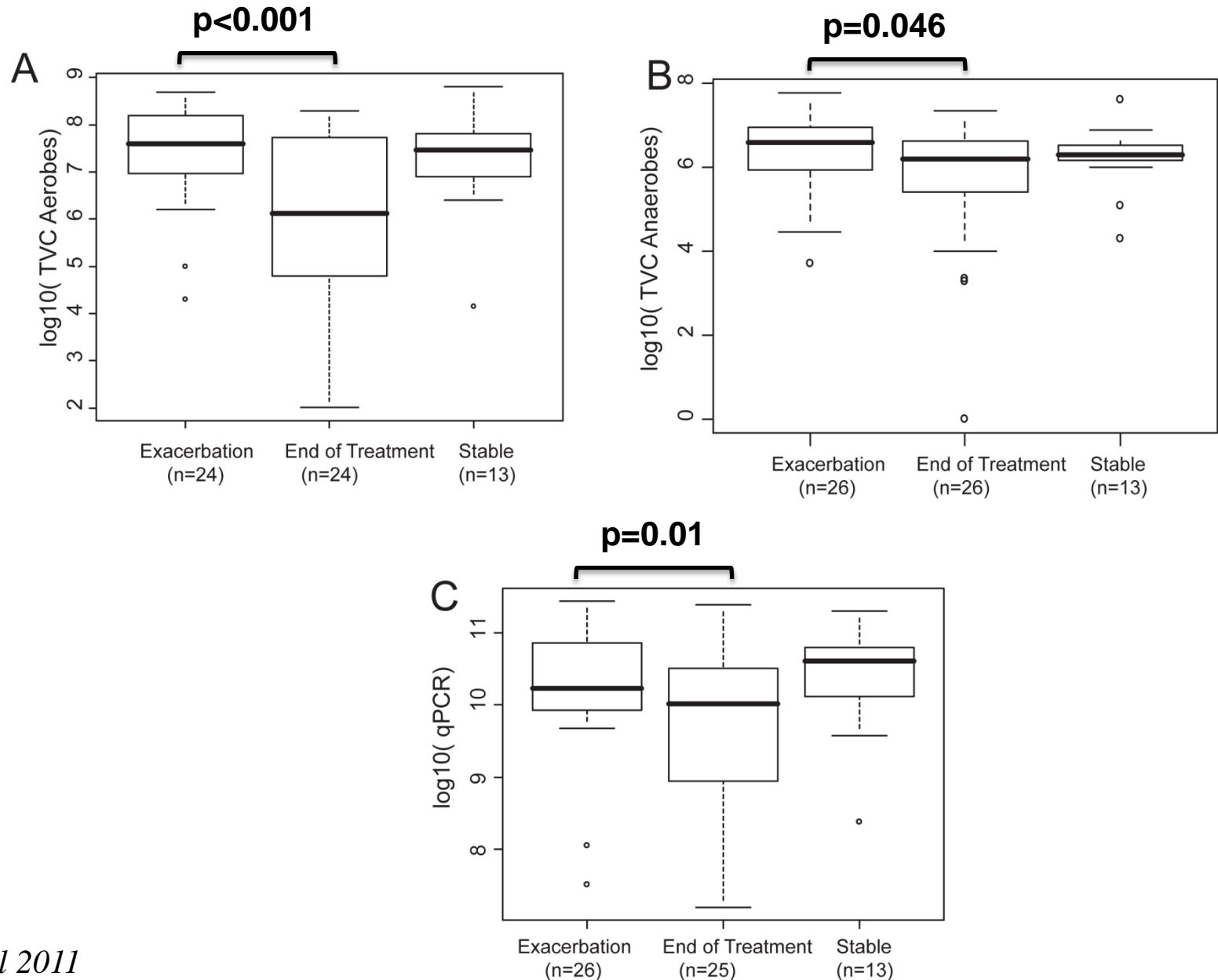


# Exacerbations and anaerobes

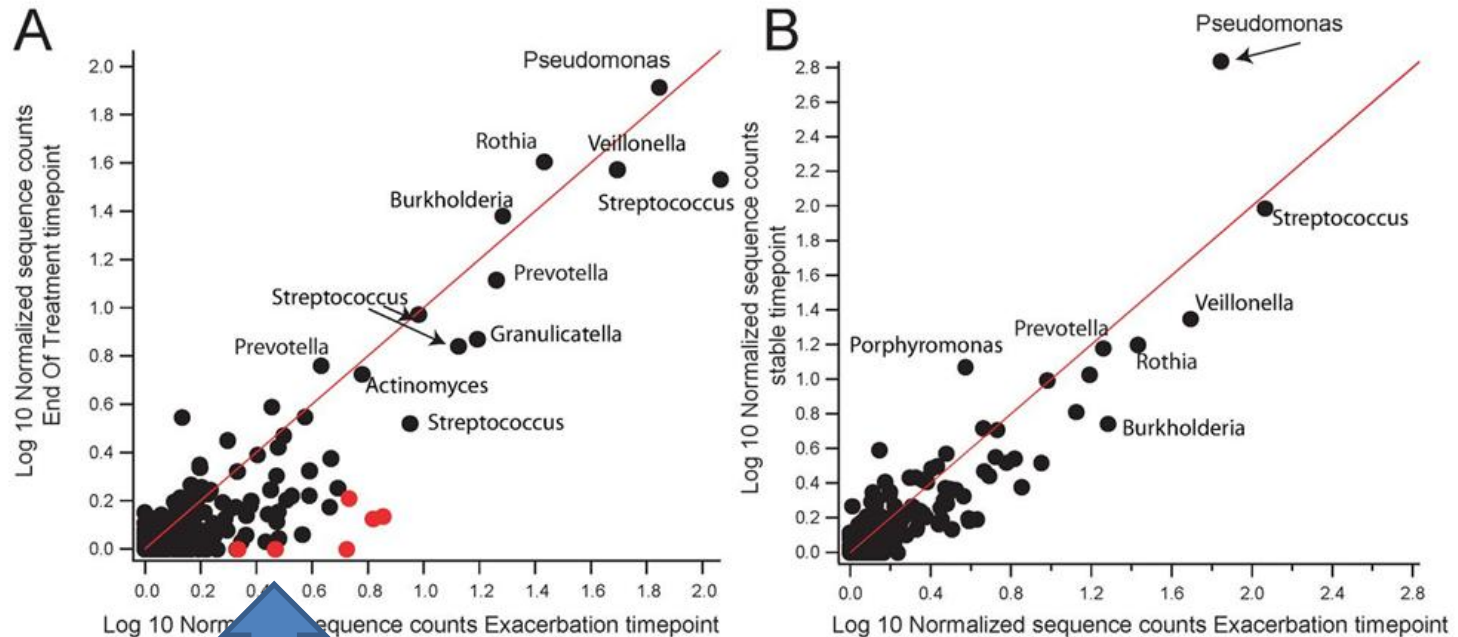
## *Lung function and inflammatory biomarkers*



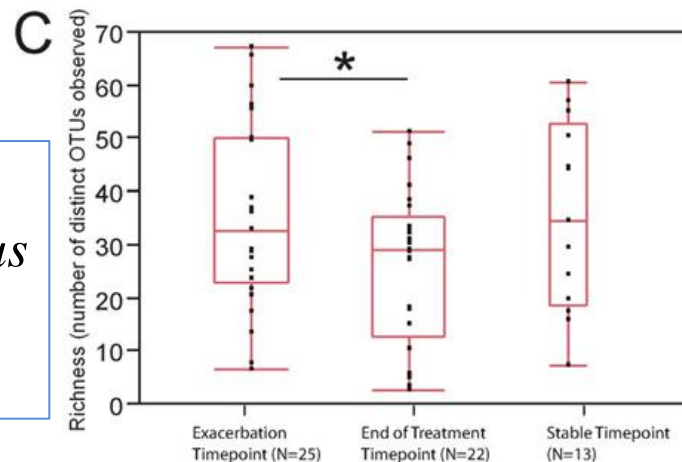
# Small effect on bacterial load



# Changes in OTUs with exacerbation treatment



*Gamella*  
*Streptococcus*  
*Pasturella*  
*Neisseria*



# Identification of new targets for therapy

Anaerobes (obligate and facultative) and/or “normal oropharyngeal flora” - often present in very high densities

Tunney MM, *et al. Am J Respir Crit Care Med* 177:995. 2008

✓ *Prevotella*, *Veillonella*, *Propionibacterium*, *Actinomyces*:

- high levels in BAL and sputum; lower numbers and different species in healthy adults

Ulrich M, *et al. Thorax*. 65:978. 2010

✓ *Prevotella intermedia*:

- high levels in sputum; virulence *in vitro* and *in vivo* models; serological response.

Sibley CD, *et al. Proc Natl Acad Sci USA* 105:15070. 2008.

✓ *Streptococcus milleri* group:

- associated with poor outcomes in adults



# Identification of new targets for therapy

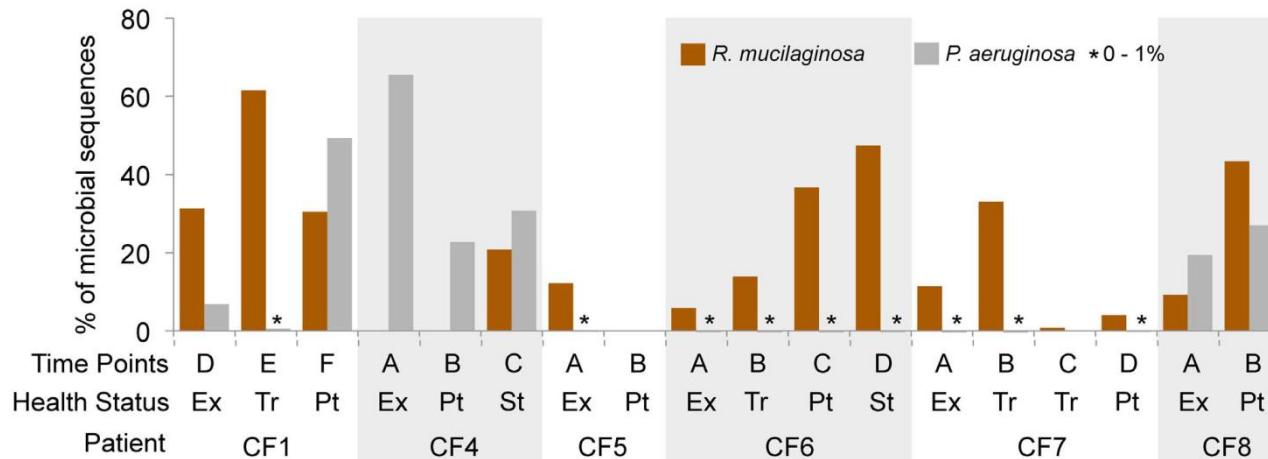
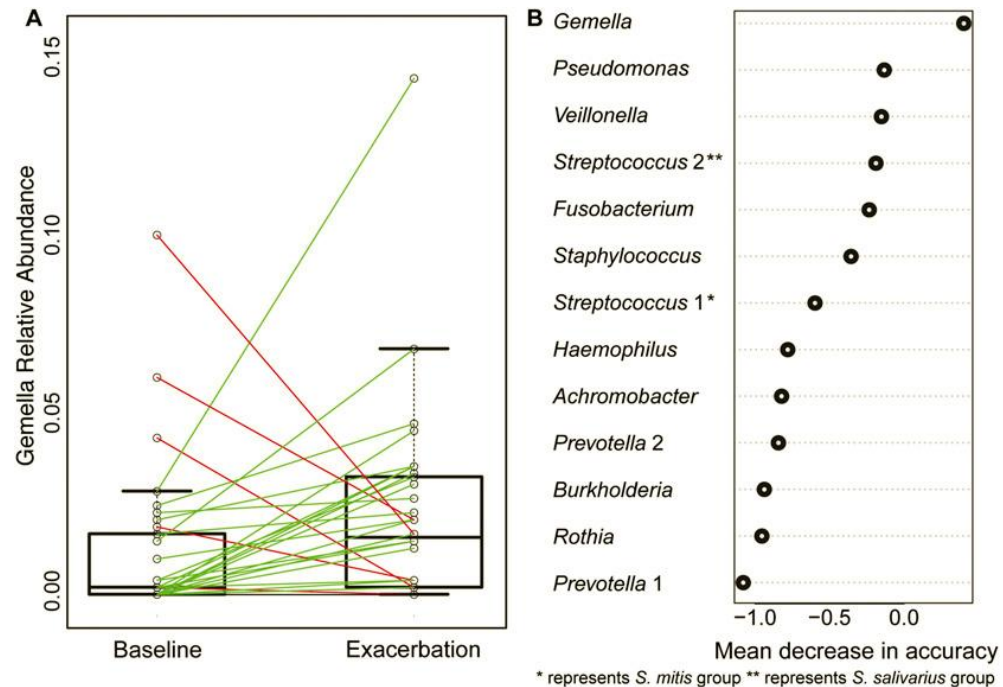
## Other unusual species

Carmody LA, *et al. Ann Am Thor Soc* 10:179. 2013

- ✓ *Gemella*: 34 baseline-exacerbation pairs

Lim YW *et al. PLoS ONE* 2013

- ✓ *Rothia mucilaginosa*: detected in 83% of patients; often in higher abundance than *P. aeruginosa*



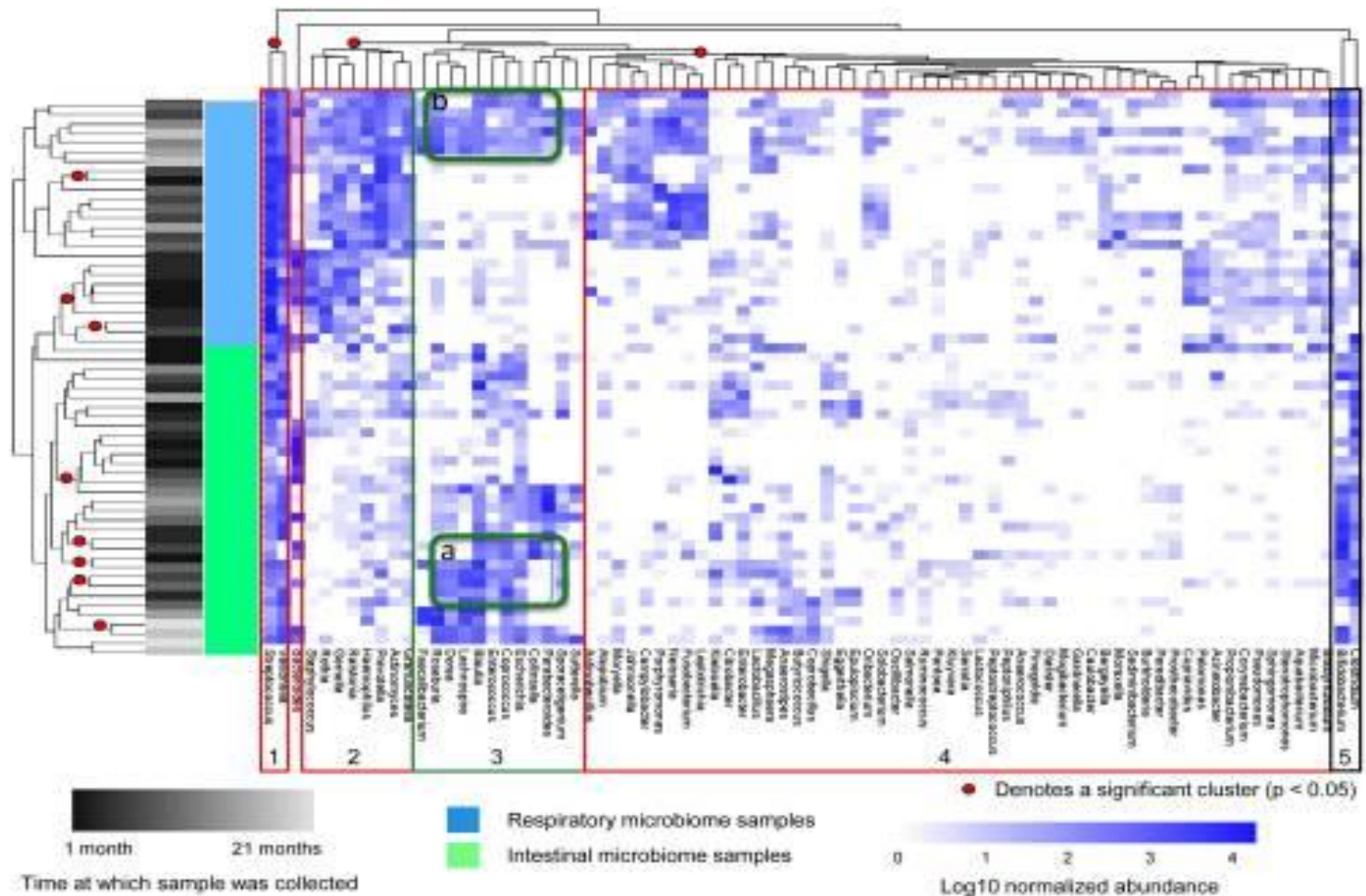
# The Respiratory Microbiome: Implications for Therapy

1. Complex airway communities may help explain why susceptibility testing of only a few species doesn't correlate well with clinical outcome
  - there is a lot more stuff down there that is affected by therapy and could (should?) be taken into account when considering therapy
2. Some 'atypical' species are found with high frequency and in high abundance
  - biomarkers of exacerbation?
  - targets for therapy?

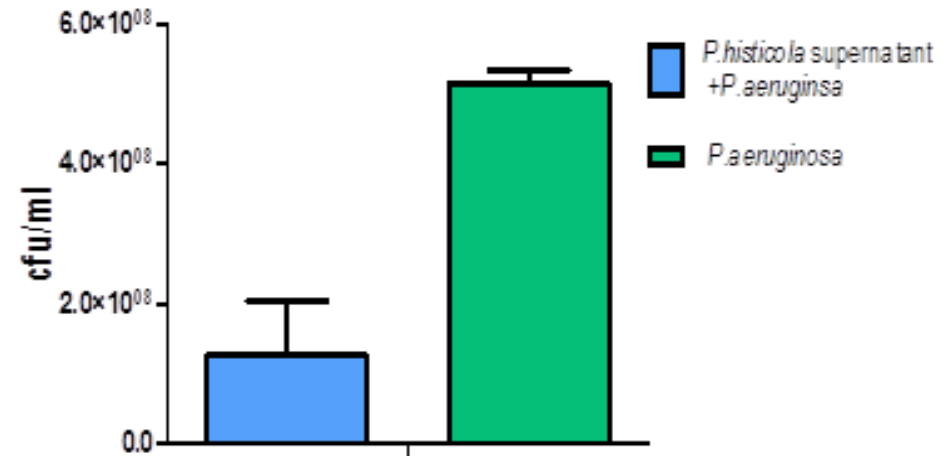
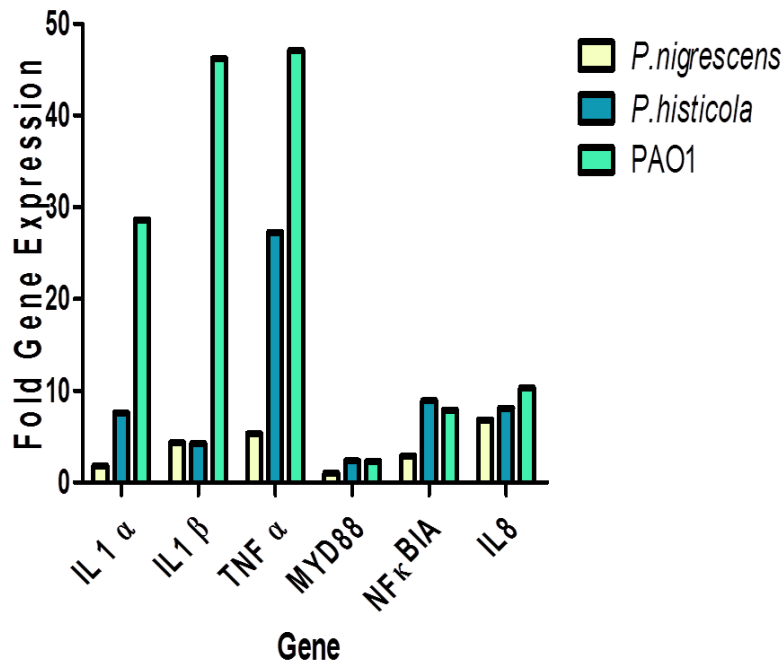
# The Respiratory Microbiome: Implications for Therapy

3. Tracking dynamics (and activity) of airway microbiota may offer refined approaches for antibiotic management
  - earlier treatment of exacerbation
  - guiding end of therapy
4. Dominant species may decrease with exacerbation
  - are we targeting the right species?
5. Diversity decreases with disease progression
  - is there a way to manipulate the community to maintain a 'healthier' structure?

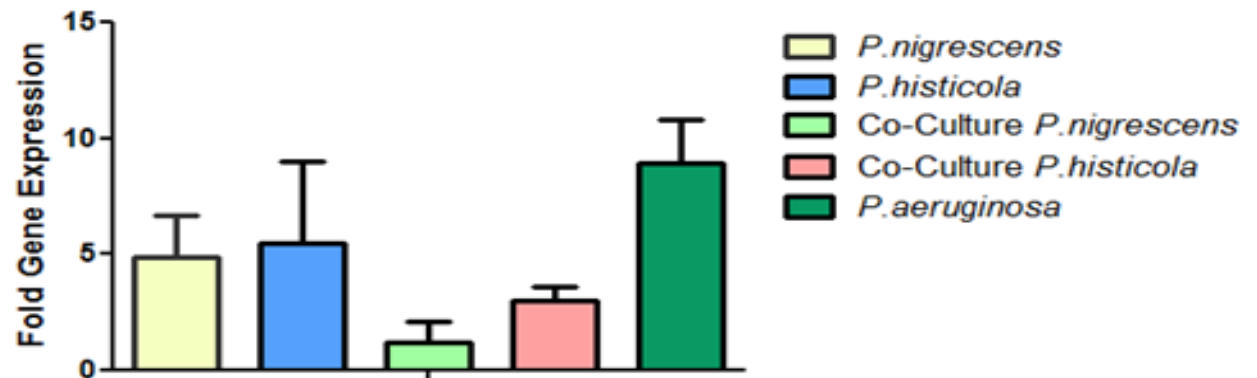
# Heat map of the intestinal and respiratory microbiome samples.



# Back to biology.....



IL-8





# Summary

- The airway in CF is a polymicrobial environment and more complex than previously thought
- Microbial diversity in the airway might be good
- Anaerobes are commonly found in CF sputum from patients when stable and during exacerbations
- Virulence of anaerobic/other bacteria has yet to be established
- Understanding the complexities of the interactions between bacteria/host/ environment is key to the development of more effective therapies for infection in CF.

# Acknowledgements

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Research  
Council

Leading science for better health

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and Learning**  
[www.delni.gov.uk](http://www.delni.gov.uk)

**Crescere non potest ergo dicere possumus nanes**

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