Carnegie Mellon - University of Pittsburgh
Ph.D. Program in Computational Biology

Comparison of the Respiratory Microbiome in Healthy Nonsmokers and Smokers

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Morris, A., Beck, J. M., Schloss, P. D., Campbell, T. B., Crothers, K., Curtis, J. L., ... & Weinstock, G. M. (2013). Comparison of the respiratory microbiome in healthy nonsmokers and smokers.*American journal of respiratory and critical care medicine*, *187*(10), 1067-1075.

Background & Goal

Compare the microbiome of the upper and lower respiratory track in healthy HIV-uninfected nonsmokers and smokers. (Lung HIV microbiome project)

- Culture-independent methods are not reliable. -> methodological challenges
- Prior work has detected bacterial DNA in cigarettes. -> direct impact

Participants → Sample Collection → Processing

Exclusion criteria

- Many criterions…

- Nonsmokers: having smoked less than 100 cigarettes in their lifetime with none/illicit inhaled drugs/cigar/pipe in the past year

- Smokers: currently smoking at least 6 cigarettes per day for at least six months and might also be smoking illicit drugs, cigars and/or pipes.

8 cities, 64 people

TABLE 1. CHARACTERISTICS OF PARTICIPANTS

Demographics	Nonsmoker (n = 45)	Smoker (n = 19)	*P* Value
Age, yr	43.1 ± 13.17	43.8 ± 10.57	0.82
Sex			0.038
Male	23 (51.1)	15 (78.9)	
Female	22 (48.9)	4 (21.1)	
Ethnicity			0.31
Hispanic	5 (11.1)	0 (0)	
Not Hispanic	40 (88.9)	19 (100.0)	
Race			0.53
White	35 (77.8)	13 (68.4)	
Other	10 (22.2)	6 (31.6)	

Data are presented as n (%) or mean ± SD.

Participants → Sample Collection → Processing

Oral washes (OW), 10ml sterile 0.9% saline

Sterile saline in a sample Collection cup

Bronchoalveolar lavage (BAL)

10ml to 50ml of sterile 0.9% saline washed through bronchoscope

- Gargle with 10ml to 50ml of sterile before topical anesthesia
- The bronchoscope is then inserted (a maximum of 300 ml 0.9% saline)

Participants → Sample Collection → Processing

6 centers for DNA extraction
1 center for DNA sequencing at Washington University

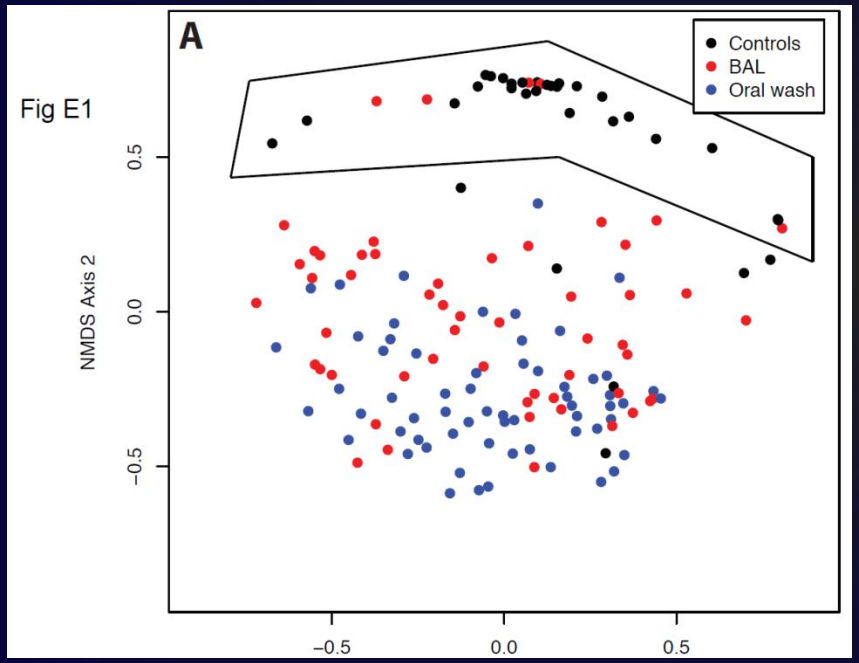
- DNA extraction validation (5 BAL specimens/center)

  The amount of amplified material doesn't correlate with the center but correlate with the BAL sample.

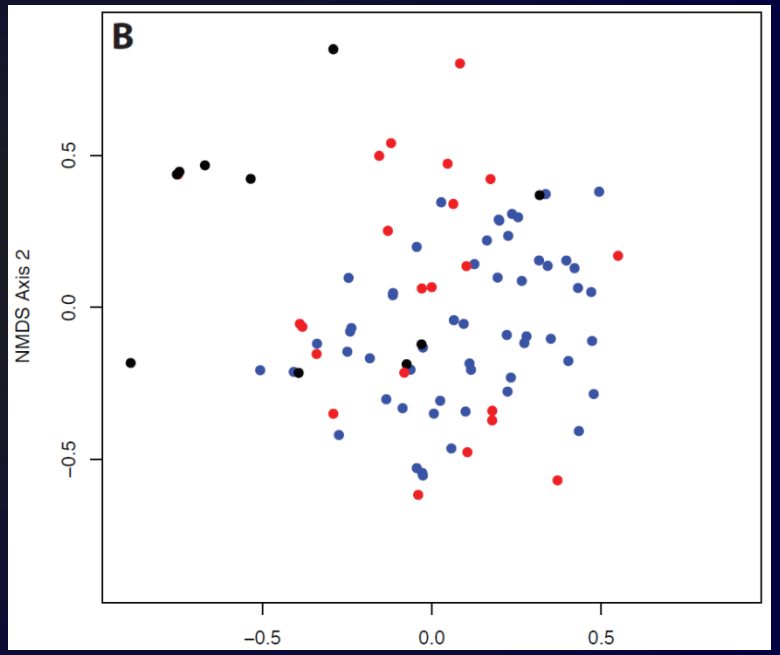
- Negative control (reagent –derived contamination)
  V1-V3: Several BAL samples have similar community structures compared with samples
  V3-V5: No significant overlap

Participants → Sample Collection → Processing

V1-3



V3-5



Non-metric multidimensional scaling

Participants → Sample Collection → Processing

- 16S rRNA gene sequences (highly conserved)

  Two variable regions (separate) are amplified, which are V1-3 (base 27 to 534) and V3-5 (base 357 to base 926)

- OTU (Operational Taxonomic Units)
  Definition: A cluster of reads with 97% similarity.
  Cutoff: 0.03 distance

- **Mothur package**

Analysis

Confounding factors

- Sex
  Repeat analysis comparing OW and BAL in nonsmokers and smokers, with women excluded

- The degree of smoking
  Split the participants based on median pack-year smoking history

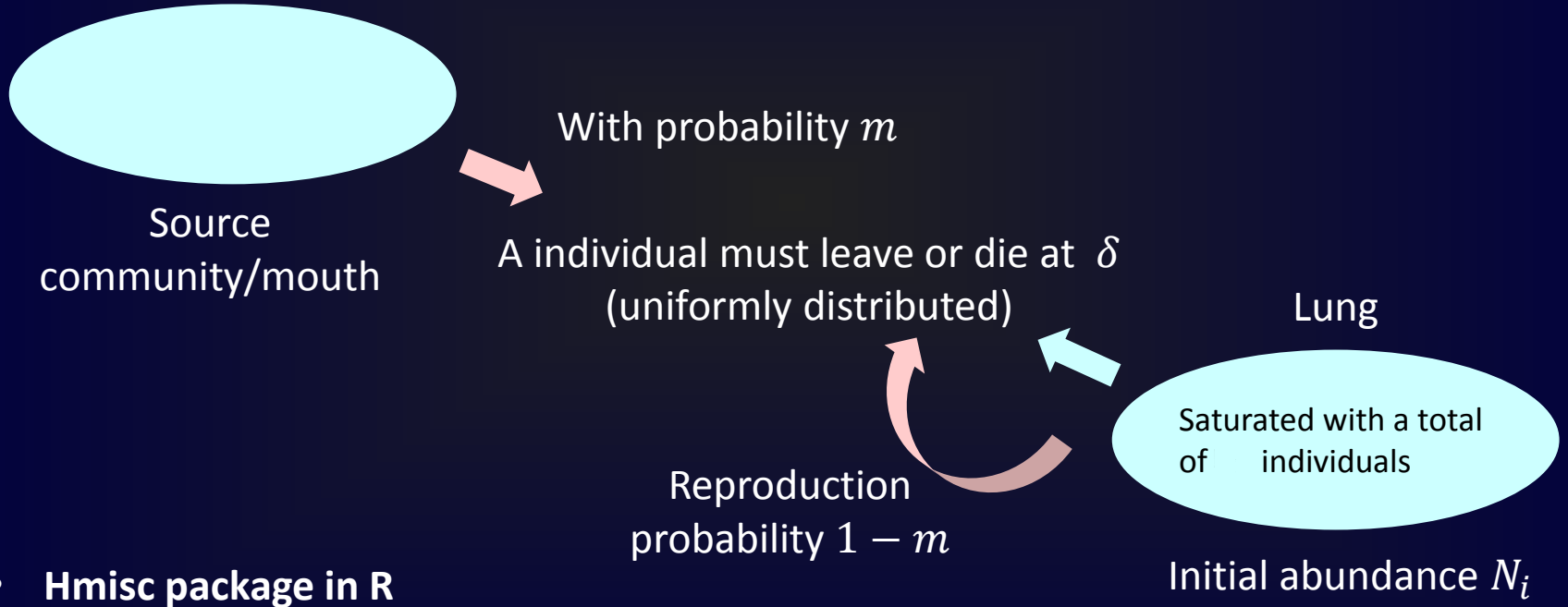
- Body mass index (BMI)
  Compare diversity measure between participants categorized by BMI

- Systematic differences between centers

Analysis

Is mouth a source for the microbial community in the lung?

- Neural model



- **Hmisc package in R**

Sloan, W. T., Lunn, M., Woodcock, S., Head, I. M., Nee, S., & Curtis, T. P. (2006). Quantifying the roles of immigration and chance in shaping prokaryote community structure. *Environmental microbiology*, *8*(4), 732-740.

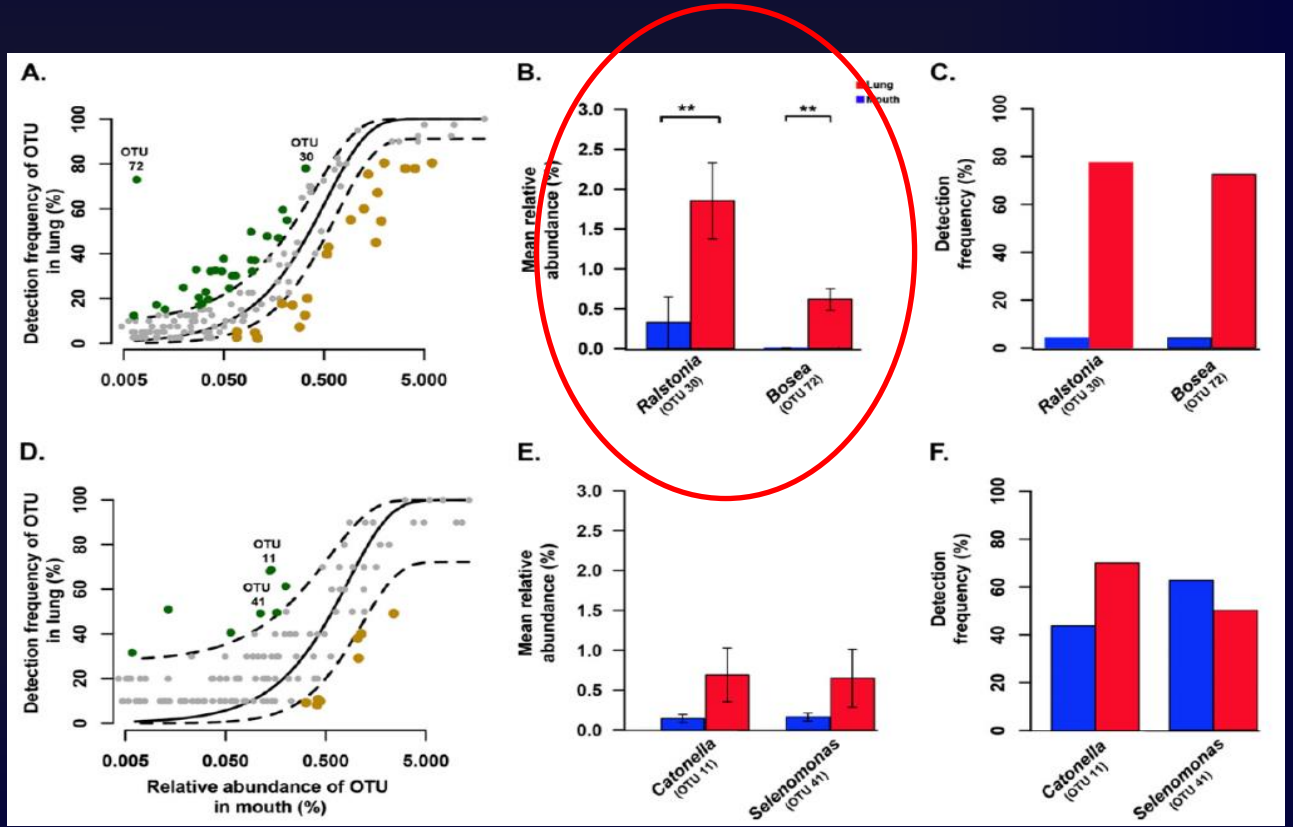
Result

1. Mouth as a source explains much of the microbial community in the lungs

V1-3

nonsmokers

smokers



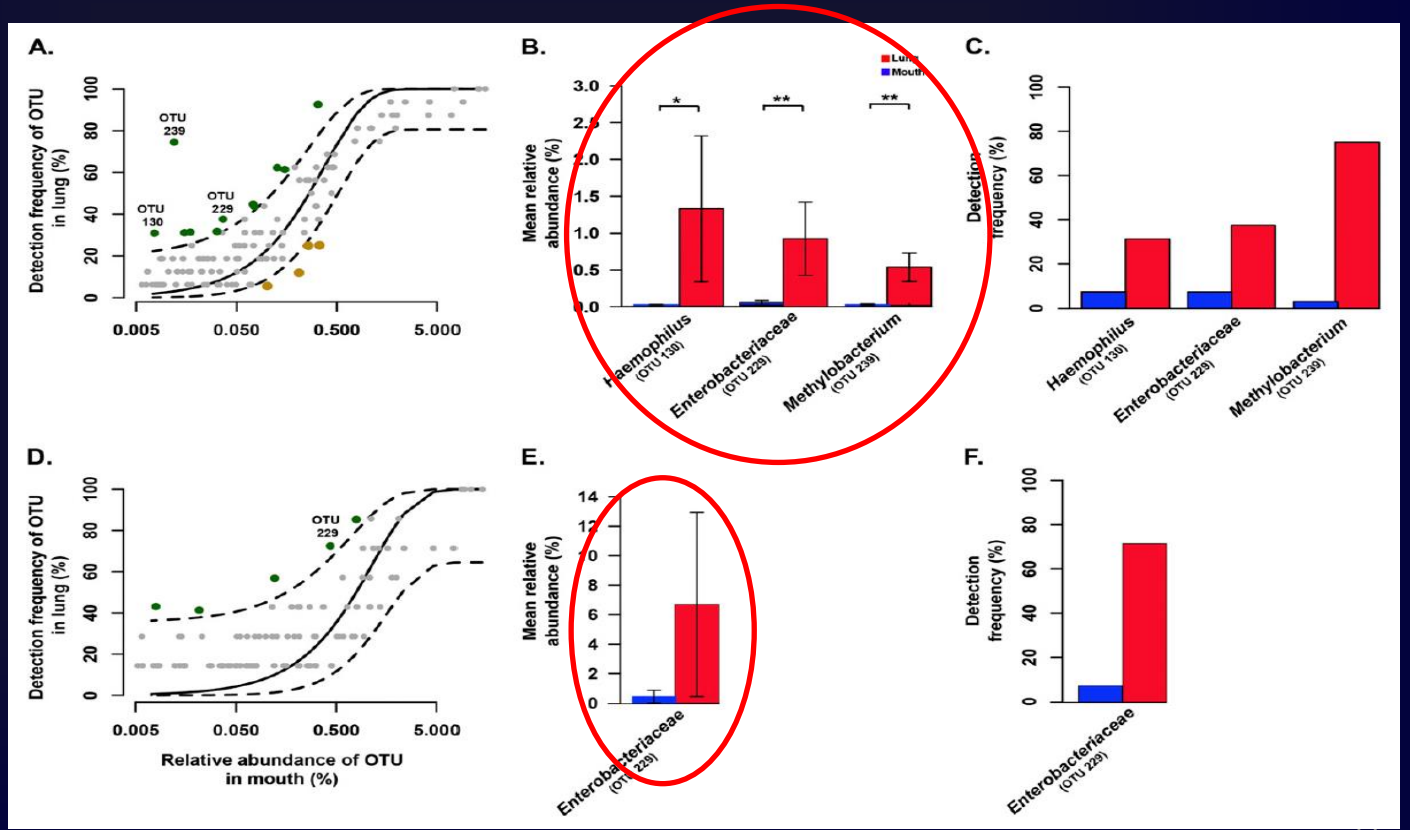
Result

1. Mouth as a source explains much of the microbial community in the lungs

V3-5

nonsmokers

smokers

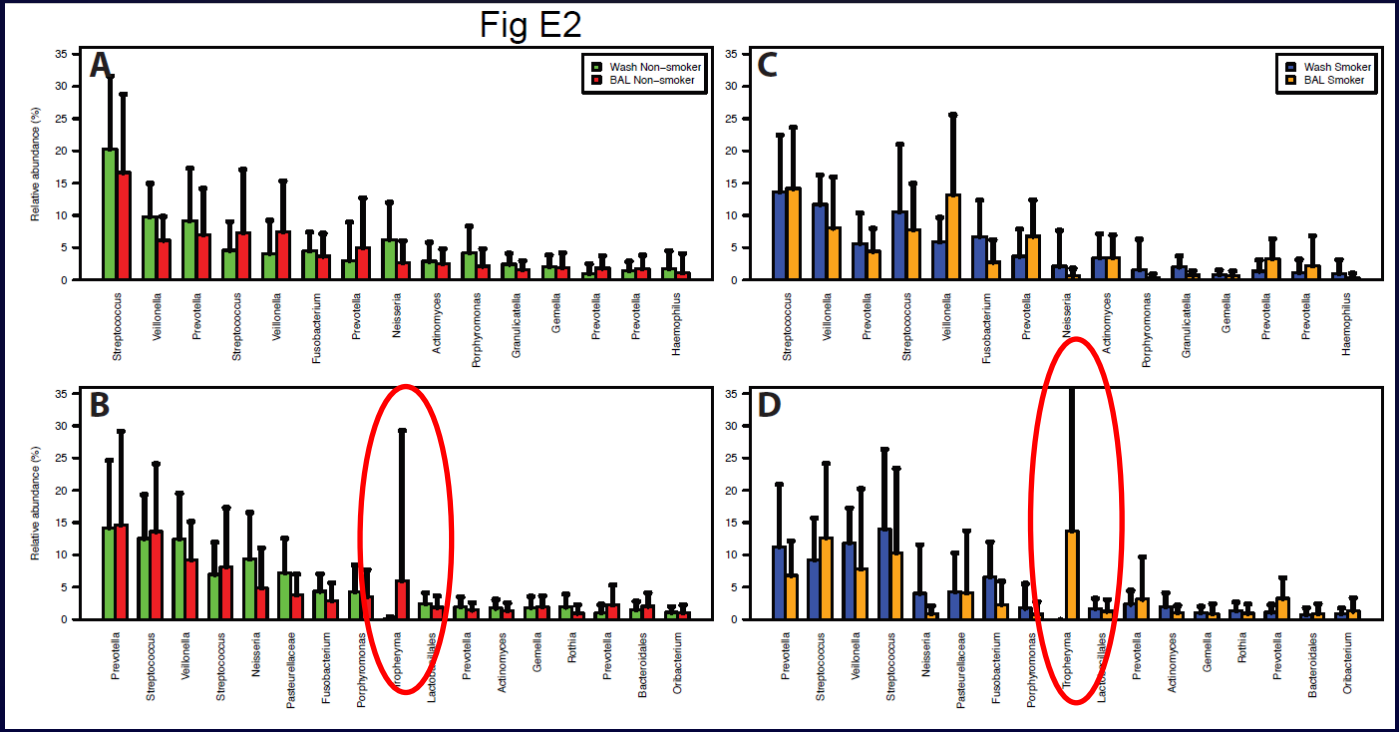


Result

2. Particular OTUs are differentially represented in BAL compared with OW communities

V1-3

V3-5

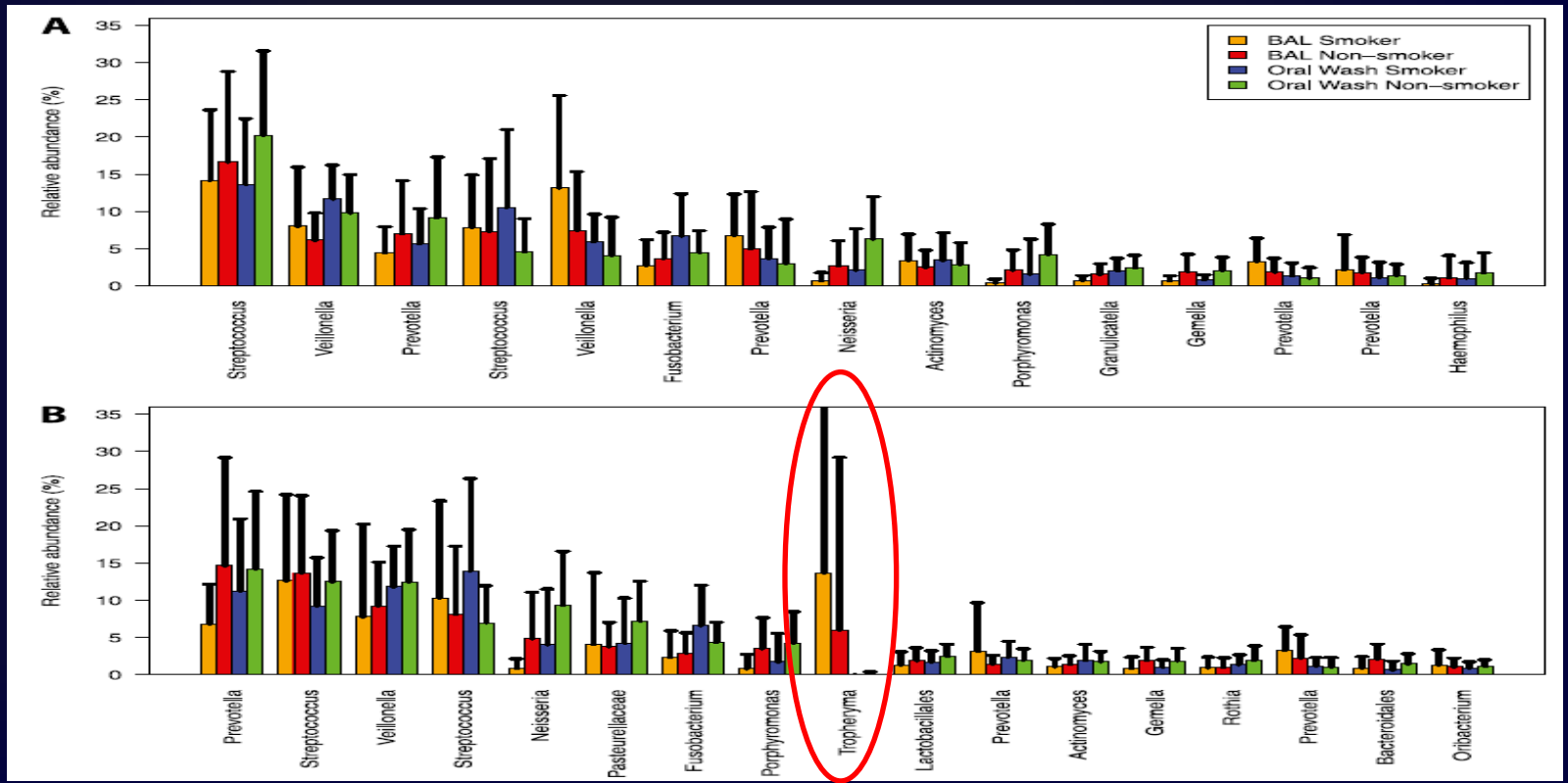


Result

2. Particular OTUs are differentially represented in BAL compared with OW communities

V1-3

V3-5

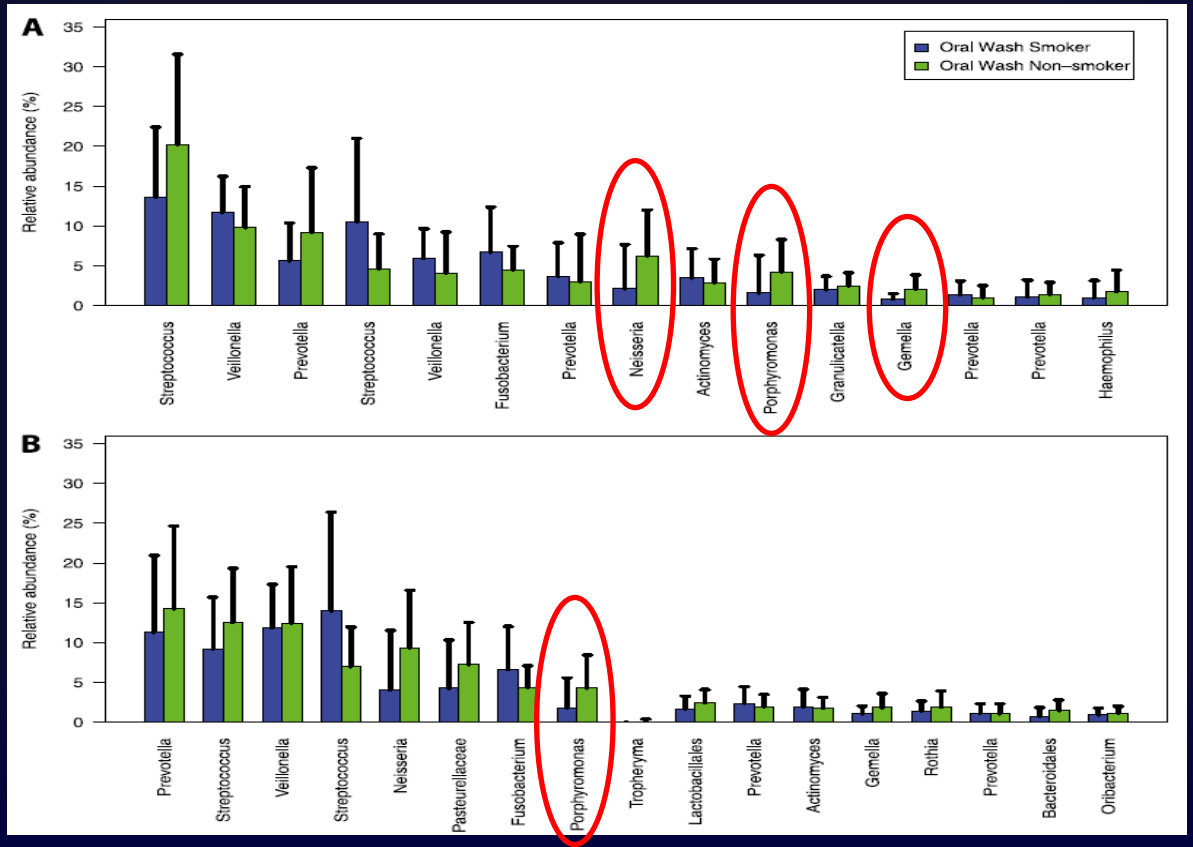


Result

3. OUT-Level Comparisons between Nonsmokers and smokers (OW)

V1-3

V3-5

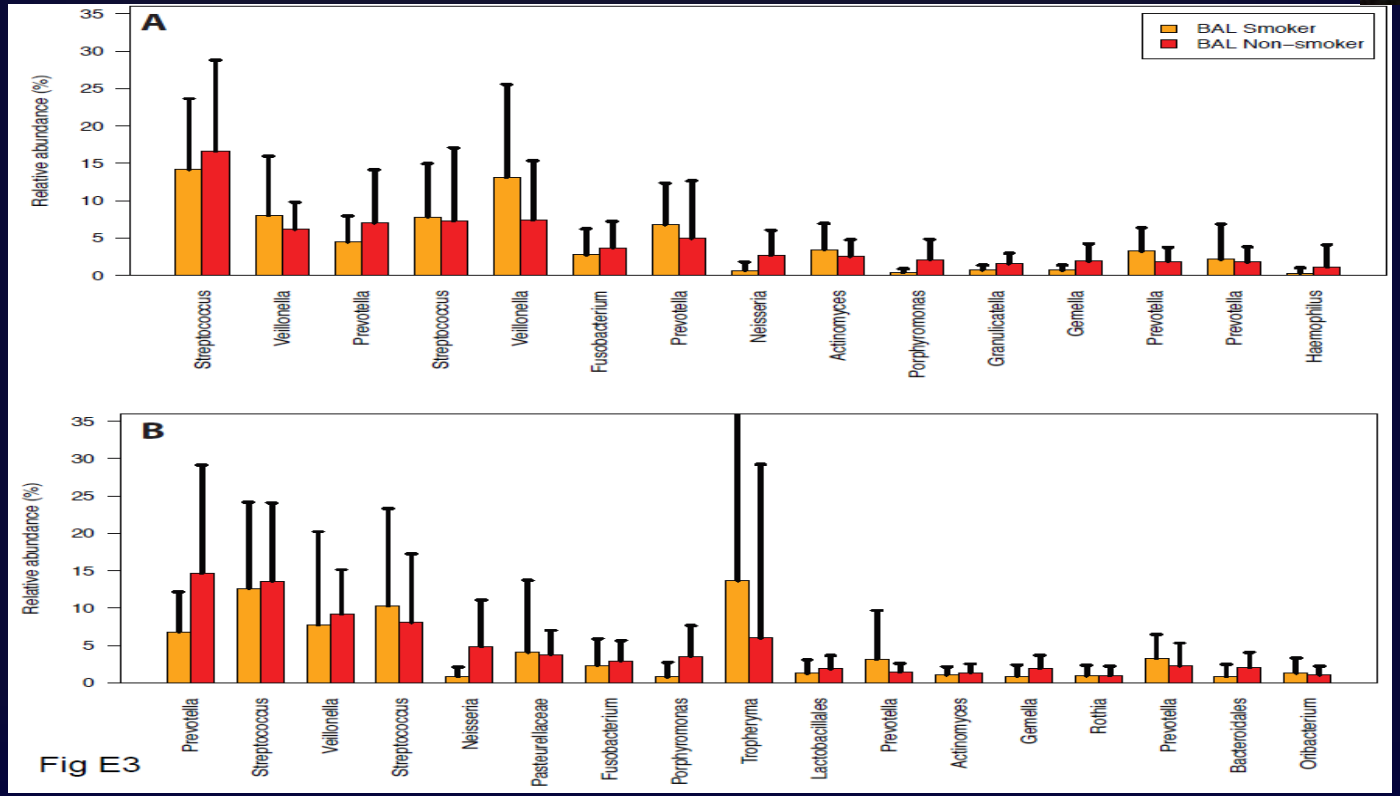


Result

3. OUT-Level Comparisons between Nonsmokers and smokers (BAL)

V1-3

V3-5



Result

4. α diversity (number of different bacterial sequences in a sample)

Region	Smoker	Site	Samples	Observed Richness	Shannon Index	Inverse Simpson Index	Phylogenetic Diversity
V1–3	No	BAL	37	58.4 (18.8)	2.90 (0.35)	10.9 (3.9)	2.26 (1.34)
	Yes	BAL	13	63.2 (28.0)	2.89 (0.49)	11.3 (5.5)	2.36 (1.52)
	No	OW	44	57.6 (13.9)	2.77 (0.34)	9.9 (3.4)	2.08 (1.41)
	Yes	OW	18	68.9 (24.0)	2.92 (0.44)	11.6 (4.4)	1.83 (1.67)
V3–5	No	BAL	16	54.0 (17.3)	2.60 (0.70)	8.8 (3.9)	2.75 (2.02)
	Yes	BAL	7	43.5 (18.1)	2.24 (1.01)	7.7 (5.6)	2.13 (1.63)
	No	OW	39	55.2 (12.8)	2.75 (0.30)	10.0 (2.9)	2.21 (1.44)
	Yes	OW	16	65.5 (18.4)	2.86 (0.46)	11.0 (4.7)	2.29 (1.39)

Definition of abbreviations: BAL = bronchoalveolar lavage; OW = oral wash; V1–3 = variable regions 1 through 3; V3–5 = variable regions 3 through 5.
All metrics are based on the average of rarefying samples to 1,000 sequences. BAL samples excluded at V1–3 and V3–5 if community structure resembled that of controls. Samples were also excluded at V3–5 if there were insufficient sequences.

- Using V1-3 regions, there are no significant effects in comparisons of smoking status or OW to BAL on any of the α diversity measure
- Using V3-5 regions, a significantly higher number of OTUs measured by V3-5 in smokers' BAL and OW than nonsmokers' ($p = 0.02$)

Result

5. Structures

V1-3

Significant overlaps between OW and BAL.

V3-5

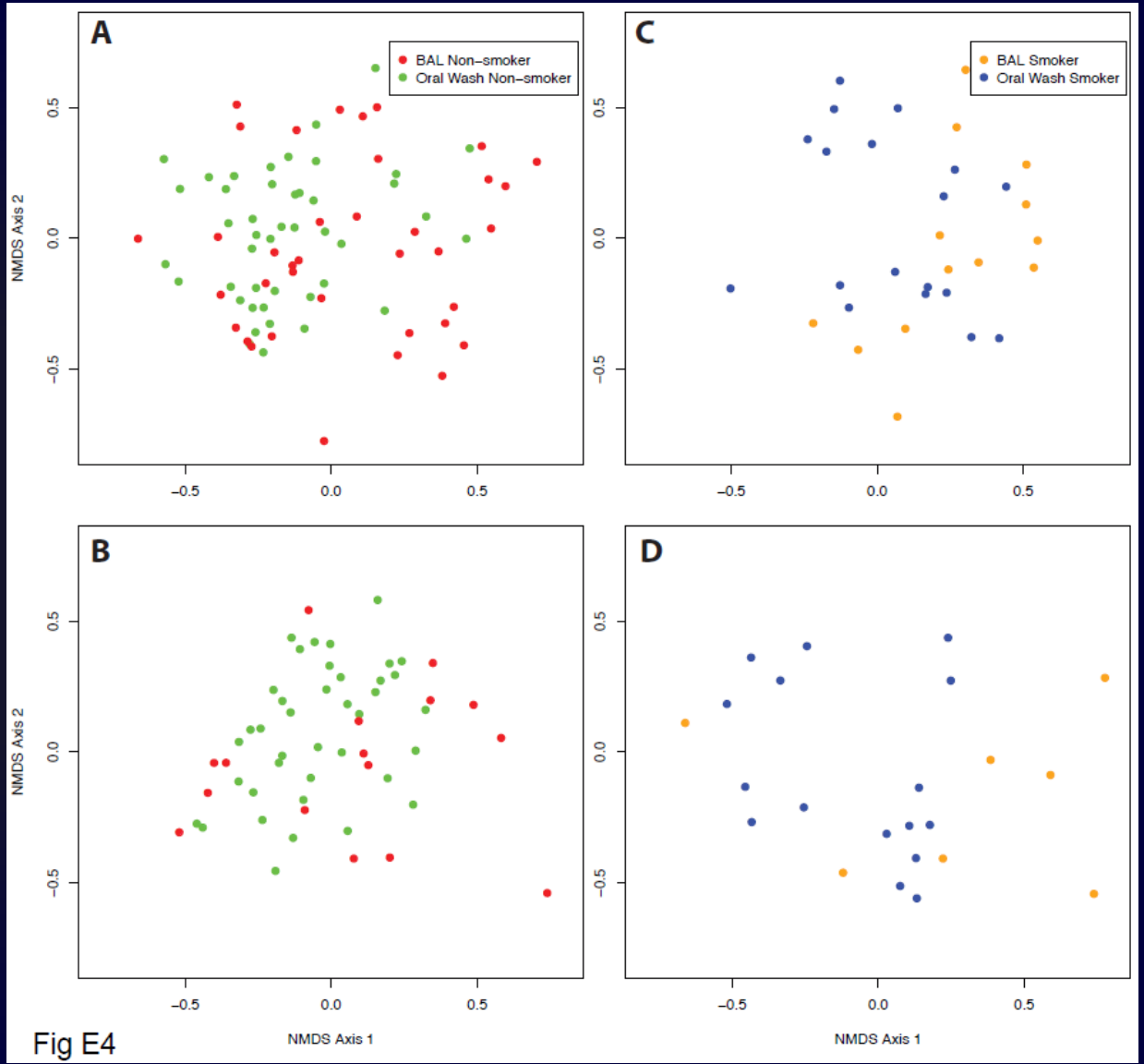


Fig E4

Result

V1-3

5. Structures

Significant differences
Among oral community,
But not in the lung
Community.

V3-5

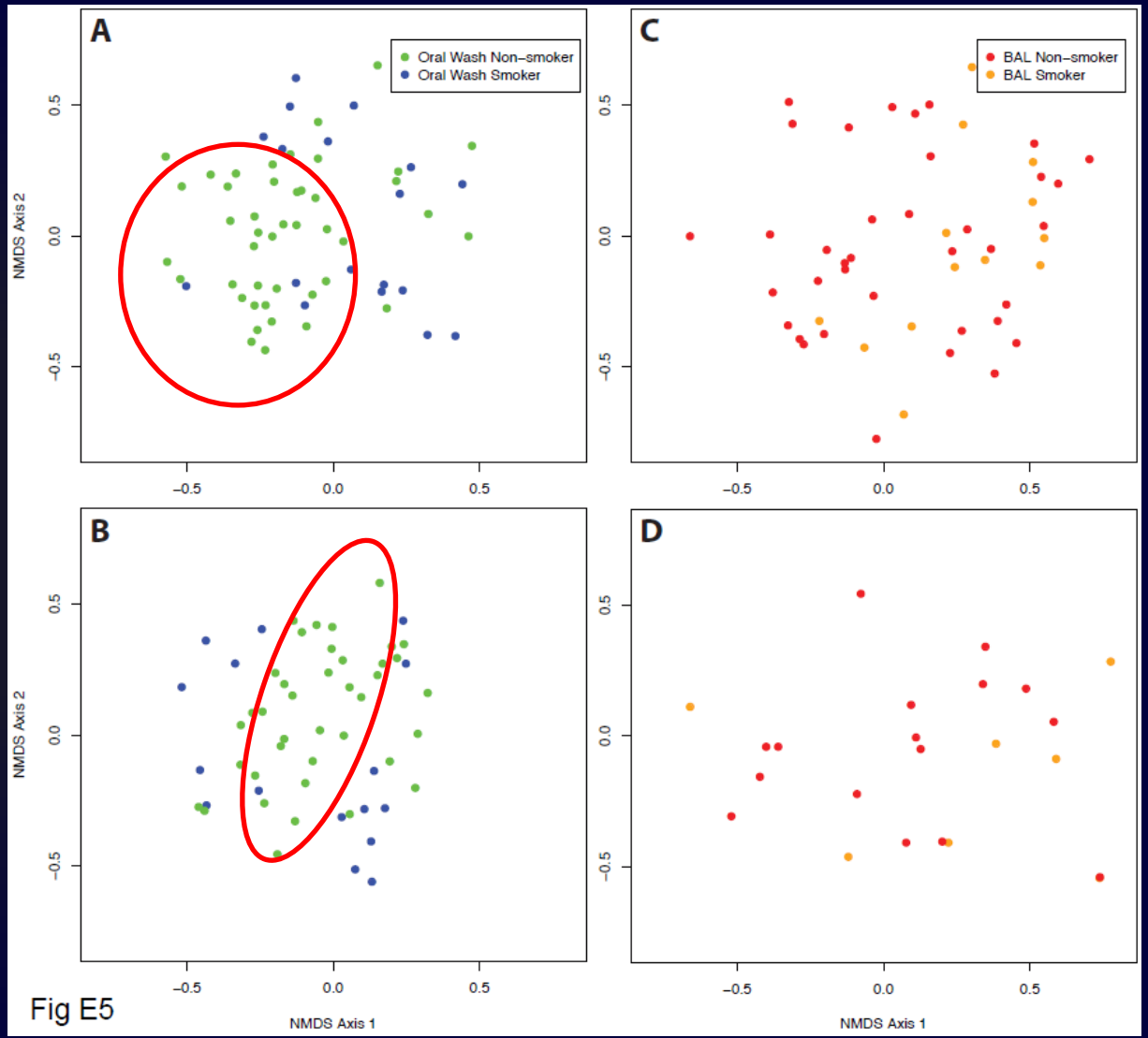
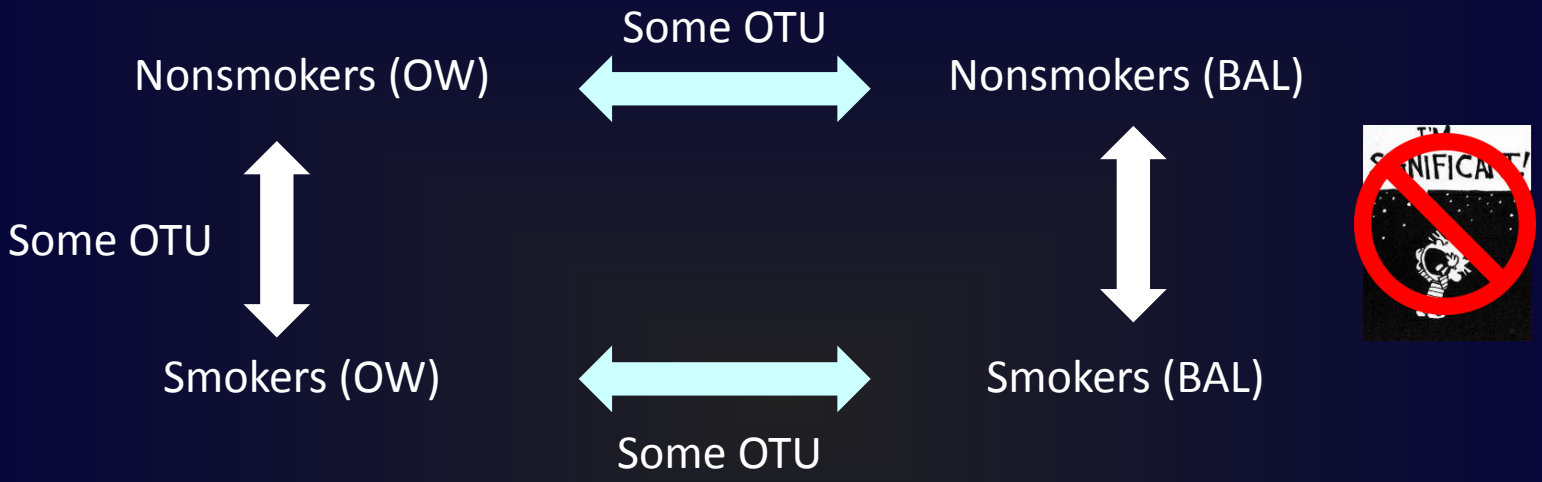


Fig E5

Conclusion



- Smoking disrupt the normal structure community structure in mouth.
  Fox example, *porphyromonas*, a bacteria linked to periodontal disease, is depleted in OW of smokers.

- Take care of the different 16S regions for amplification
  V1-V3 (more reads), V3-V5 (better detection)

Some considerations brought by authors

- Mouth is the only source community (nose, throat, gastrointestinal tract)
- Lack power to measure significant differences considering some factors (race, sex)
- Possibility of contamination
- Different methods used at difference centers
- Two-bronchoscope method?
- Neural model -> dead bacteria may be still clinically important