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Evaluating Subgingival Microbiome After Switching from Cigarettes to Nicotine Pouches

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Disclosures

- This study was sponsored by Altria Client Services.
- All authors except the presenter were employees of, or consultants to, Altria Client Services at the time the study was conducted.

Conflicts of Interest

- B. Paster, T. Chen and H. Hasturk from The Forsyth Institute have received funding from Altria Client Services to conduct this study.
- K. Milleman, J, Milleman, A. Yoder from Salus Research, Inc., have received funding from Altria Client Services to conduct this study.
- J. Liu, J. Wang, J. Edmiston, M. Sarkar, and M. Gogova are employees of Altria Client Services.

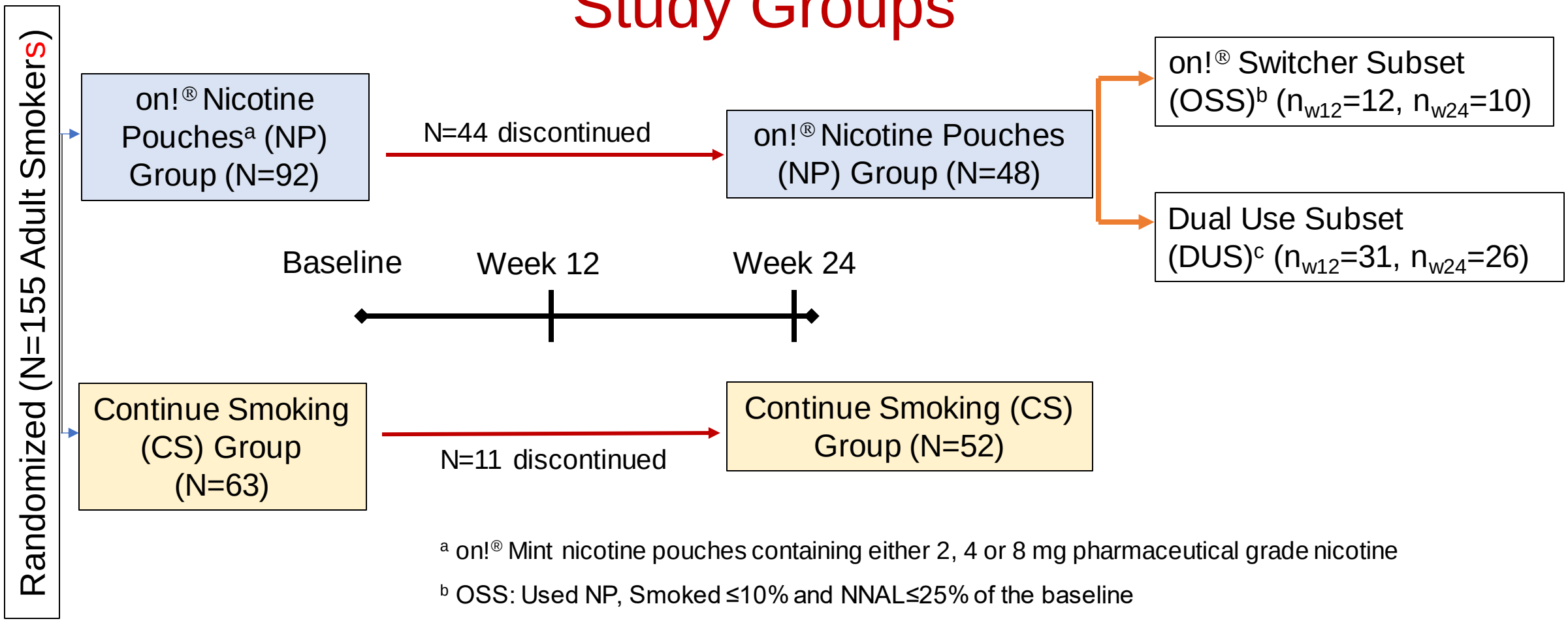
Background

- Oral disease from use of tobacco products is of significant public health concern. A consistent association between cigarette smoking and elevated oral disease (e.g. oral cancer) risks has been demonstrated relative to non-tobacco use. (*Reibel et al, 2003*)
- Combustible tobacco products confer significant risk due to harmful constituents such as carcinogens, respiratory toxicants, cardiovascular toxicants and reproductive or developmental toxicants (*Hatsukami et al, 2007)*.
- Next generation oral tobacco products, such as nicotine pouches, do not contain tobacco leaf thus many of the toxicants are substantially reduced (>95%), which may offer harm reduction opportunity for adult smokers.

Objective

The objective of this study was to compare subgingival microbiome profiles in adult smokers who switched from cigarette smoking (CS) to using on!® nicotine pouches (NPs) in a single-center, randomized, open-label, parallel-group study

Study Groups



[a] on!® Mint nicotine pouches containing either 2, 4 or 8 mg pharmaceutical grade nicotine

[b] OSS: Used NP, Smoked ≤10% and NNAL≤25% of the baseline

[c] DUS: Used NP, Smoked >10% or NNAL>25% of the baseline

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, a urinary biomarker for tobacco specific nitrosamines

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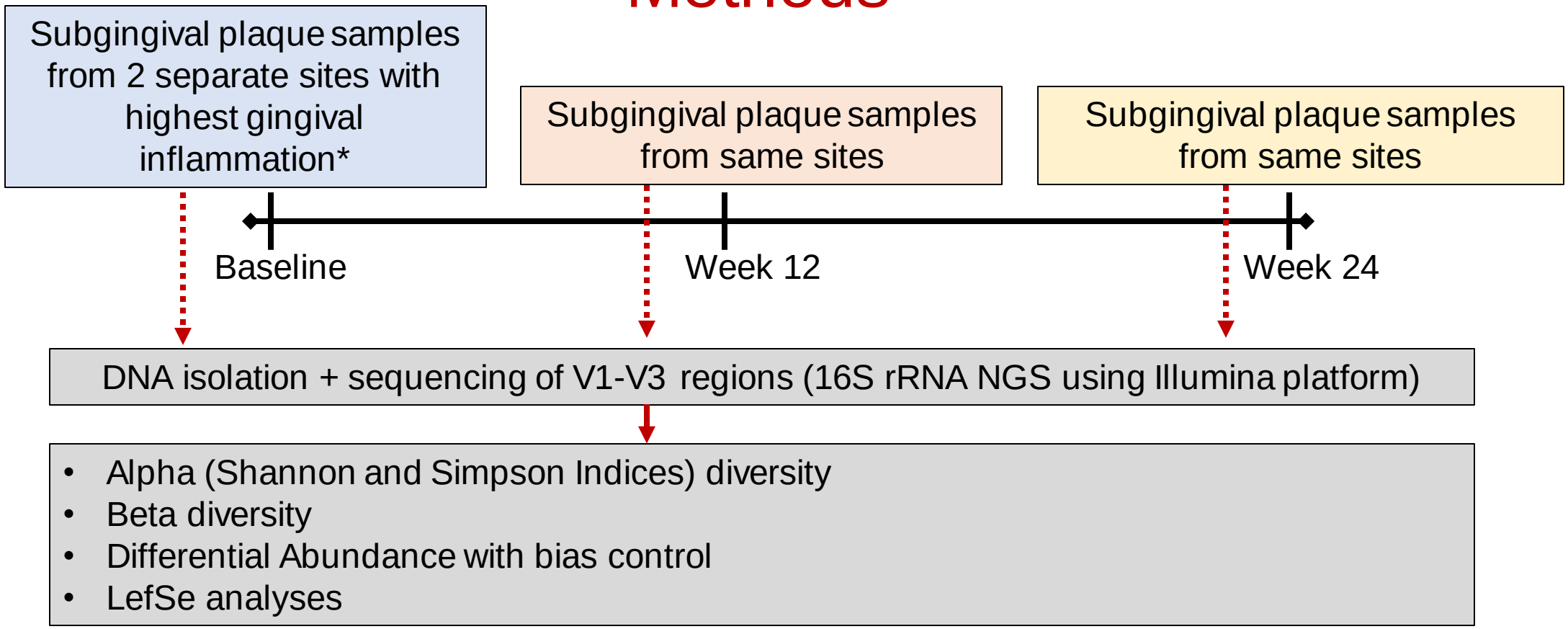
Methods



*Highest GI score and presence of marginal bleeding at baseline

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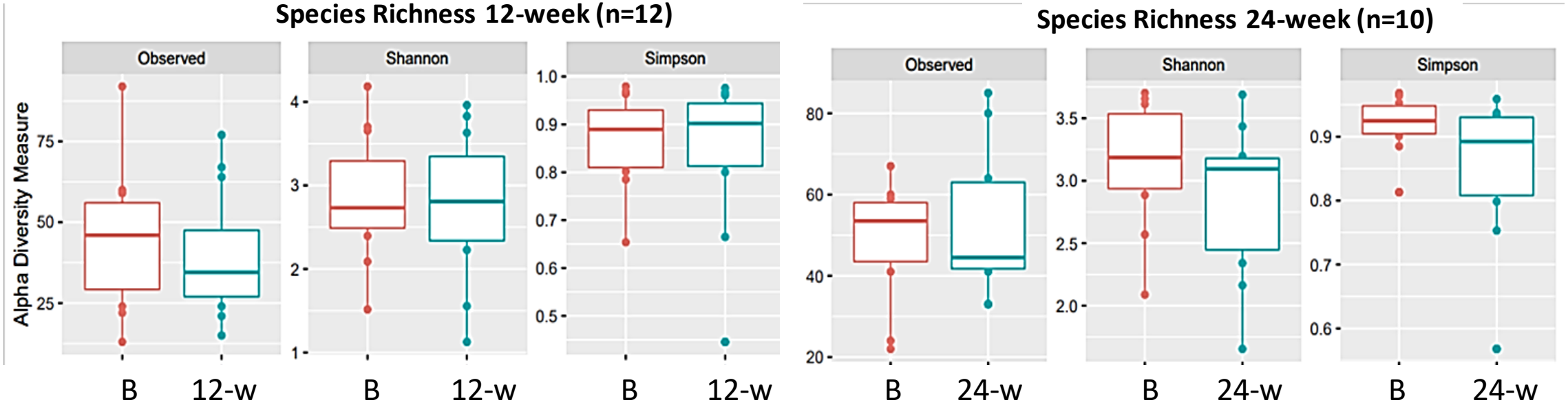
Alpha Diversities: 12- and 24-week compared to baseline

Subgroup Analysis in NP (OSS 12- and 24-w vs. Baseline)

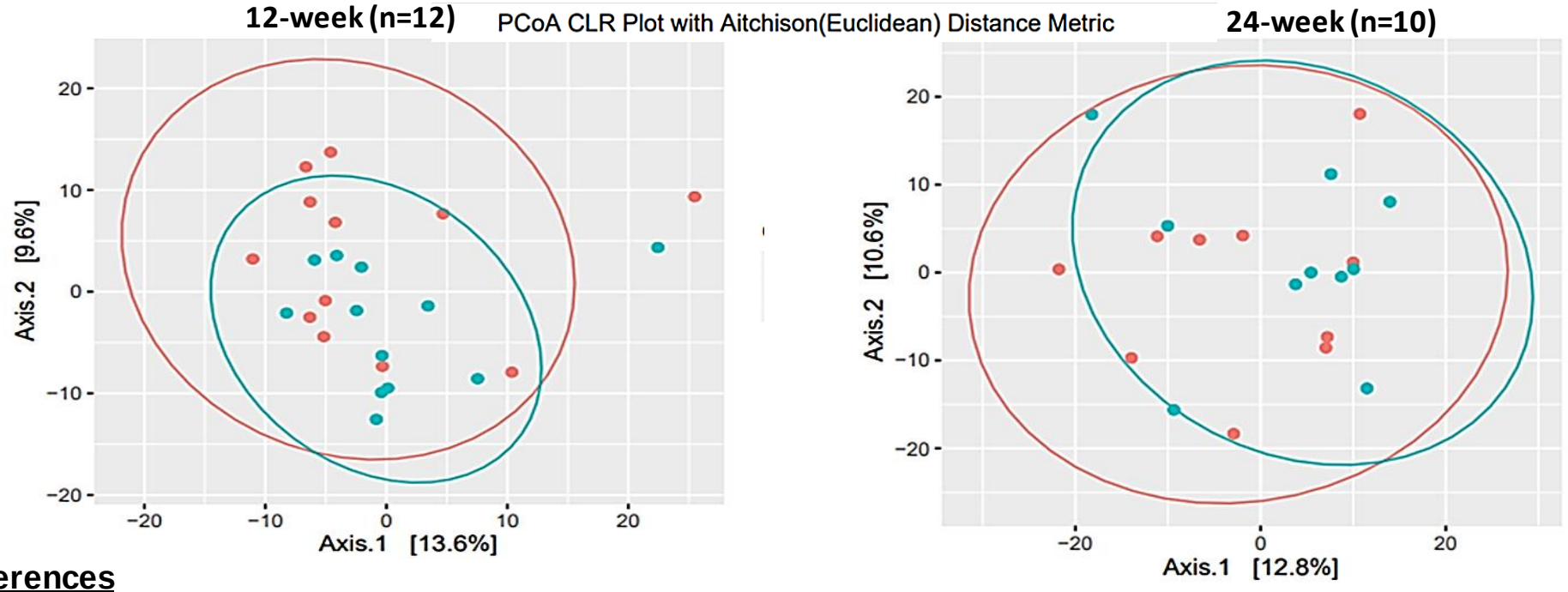


No significant differences

- Baseline vs OSS 12- or 24-week; OSS 12- vs OSS 24-week
- Baseline vs DUS 12- or 24-week; DUS 12- vs DUS 24-week
- Baseline vs DUS + OSS 12- or 24-week; DUS + OSS 12-week vs DUS + OSS 24-week
- Baseline vs CC 12- or 24-week; CC 12- vs CC 24-week

Beta Diversities: 12- and 24-week compared to baseline

Subgroup Analysis in NP (OSS 12- and 24-w vs. Baseline)



No significant differences

- Baseline vs OSS 12- or 24-week; OSS 12- vs OSS 24-week
- Baseline vs DUS 12- or 24-week; DUS 12- vs DUS 24-week
- Baseline vs DUS + OSS 12- or 24-week; DUS + OSS 12-week vs DUS + OSS 24-week
- Baseline vs CC 12- or 24-week; CC 12- vs CC 24-week

Differential Abundance with Bias Control: **OSS 12-week compared to baseline**

1	Coefficier	Standard E	Test Statis	p Values	Adjusted	Differenti*
SolobacteriumSolobacterium moorei (SP106)	-0.92022	0.510903	-1.80116	0	0	TRUE
StreptococcusStreptococcus oralis_subsp._dentisani_clade_058 (SP14	1.349733	0.655837	2.058031	0	0	TRUE
NeisseriaNeisseria flavescens (SP209)	1.375588	0.590981	2.327633	0	0	TRUE
ActinomycesActinomyces israelii (SP256)	-0.86435	0.638317	-1.3541	0	0	TRUE
RothiaRothia aeria (SP269)	2.128949	0.737517	2.886645	0	0	TRUE
FretibacteriumFretibacterium fastidiosum (SP345)	1.260529	0.513739	2.453639	0	0	TRUE
PeptidiphagaPeptidiphaga sp._HMT_183 (SP428)	-1.24142	0.754837	-1.64463	0	0	TRUE
PrevotellaPrevotella oulorum (SP57)	1.367926	0.626296	2.184154	0	0	TRUE
AlloprevotellaAlloprevotella tannerae (SP63)	1.333515	0.557101	2.393667	0	0	TRUE
CapnocytophagaCapnocytophaga sp._HMT_380 (SP75)	-1.02772	0.576538	-1.78257	0	0	TRUE
KingellaKingella oralis (SP253)	2.704444	0.795229	3.400836	0.000672	0.084647	FALSE
StreptococcusStreptococcus cristatus (SP214)	2.477068	0.875073	2.8307	0.004645	0.575934	FALSE
HaemophilusHaemophilus parainfluenzae (SP83)	2.458321	1.003883	2.448812	0.014333	1	FALSE
NeisseriaNeisseria sicca (SP215)	1.671454	0.773691	2.160365	0.030744	1	FALSE
FusobacteriumFusobacterium nucleatum (SP17)	-2.0095	1.027755	-1.95523	0.050556	1	FALSE
CapnocytophagaCapnocytophaga endodontalis (SP159)	1.345322	0.707045	1.902739	0.057075	1	FALSE
CampylobacterCampylobacter concisus (SP88)	1.255618	0.661446	1.898292	0.057658	1	FALSE
CapnocytophagaCapnocytophaga gingivalis (SP6)	1.38283	0.729578	1.895383	0.058042	1	FALSE
CapnocytophagaCapnocytophaga sputigena (SP24)	1.205182	0.669458	1.800235	0.071824	1	FALSE

- Health associated species, e.g., *Rothia*, *Neisseria*, and spp. of *Streptococcus* were more abundant (**green**) at 12 weeks as compared to baseline ($p<0.001$).
- *Kingella oralis*, was also more abundant ($p=0.08$)
- *Fusobacterium nucleatum*, a putative pathogen, and *Solobacterium moorei,* often associated with halitosis, was reduced (**red**) after 12 weeks ($p<0.001$)
- Overall results were similar at 24 weeks (data not shown)

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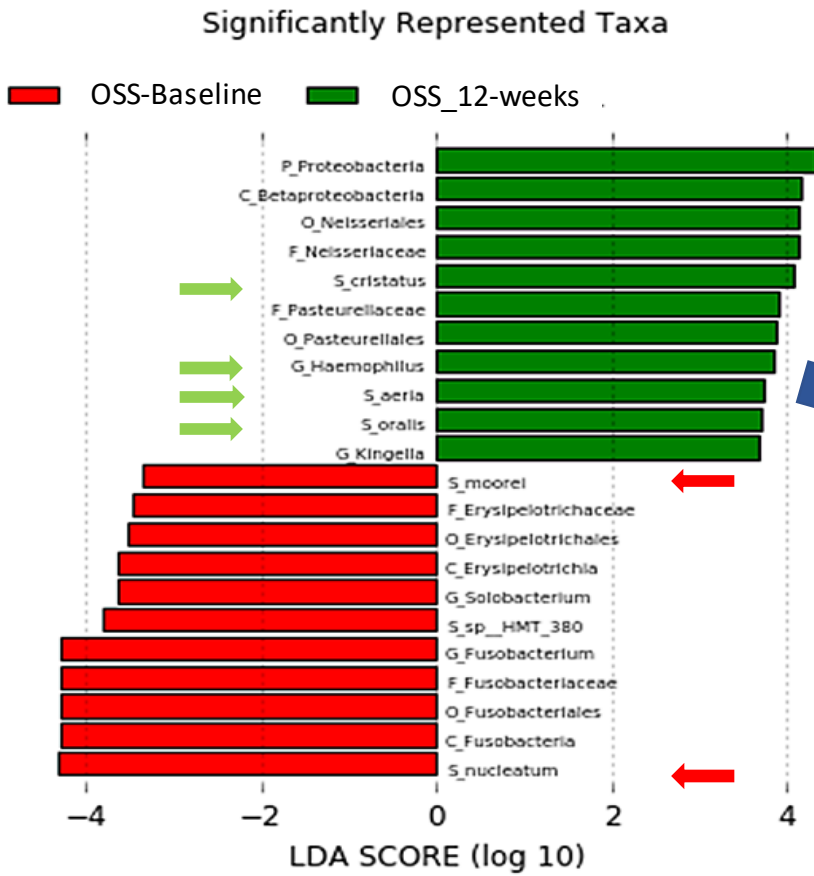
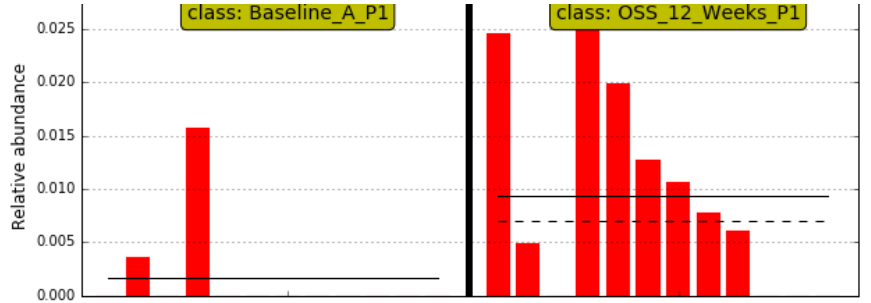
* "TRUE" = Adjusted *p*-value < 0.05; "FALSE" = Adjusted *p*-value ≥ 0.05

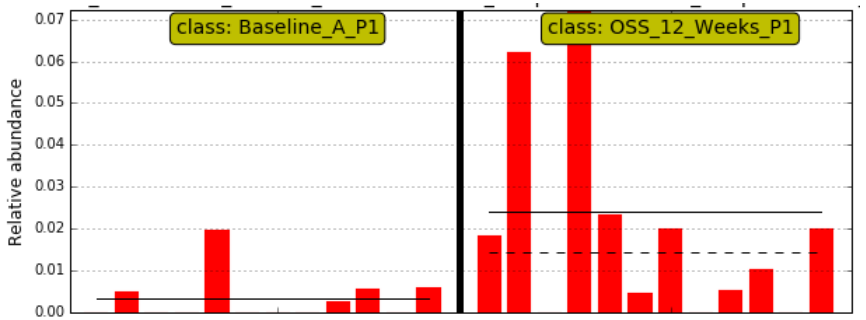
LefSe at species level: **OSS 12-week compared to baseline**



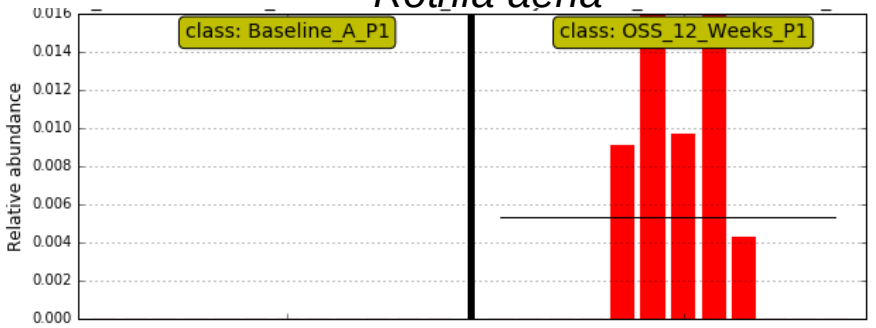
Kingella oralis



Streptococcus cristatus



Rothia aeria

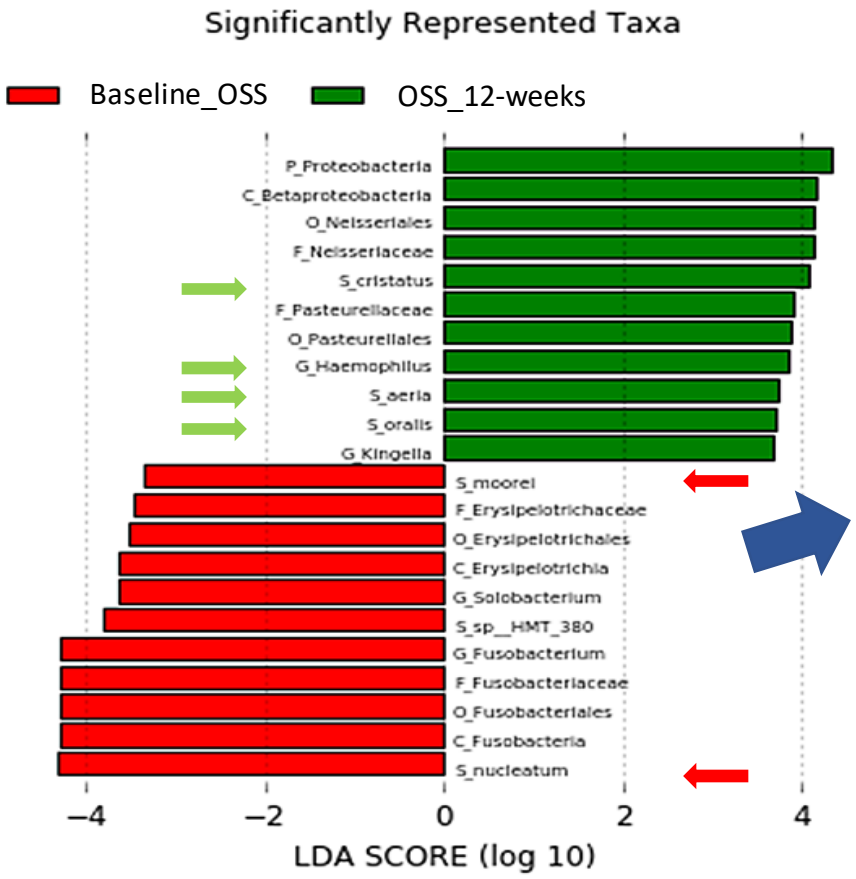


- **Health-associated species** (*Streptococcus cristatus, Kingella oralis, Rothia aeria, Haemophilus parainfluenzae*) were more prevalent at 12 weeks (Green arrows).
- Conversely, putative pathogens (*Solobacterium* and *Fusobacterium*) were reduced at 12 weeks (red arrows) and 24 weeks (data not shown).

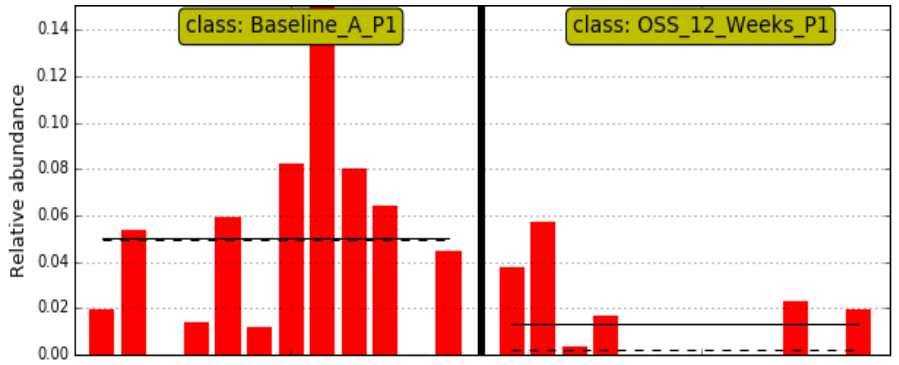
Group means

Group medians

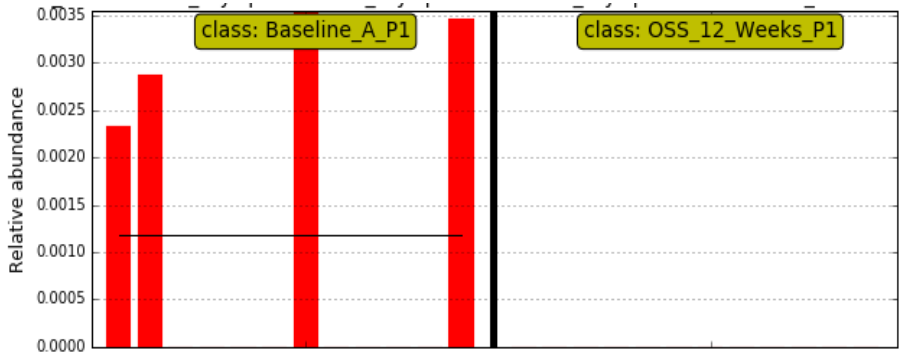
LefSe at species level: **OSS 12-week compared to baseline**



Fusobacterium nucleatum



Solobacterium moorie



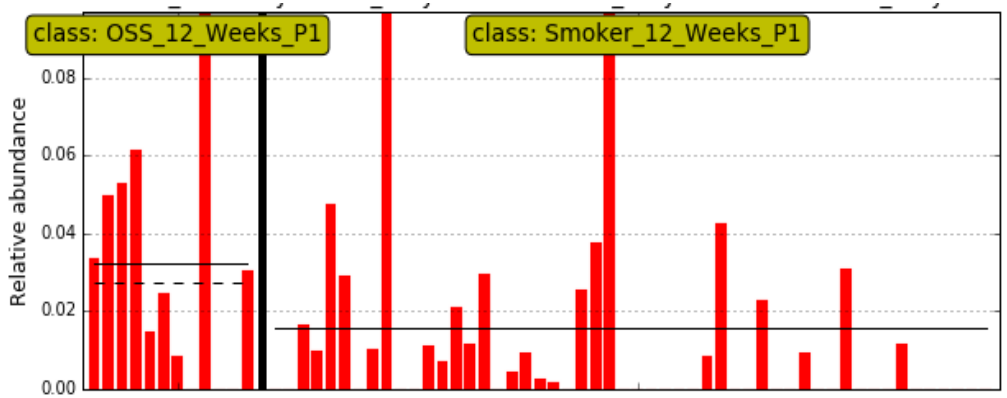
- **Disease-associated species** (*Fusobacterium, Solobacterium*) were prevalent at baseline and reduced at 12 weeks and 24 weeks (data not shown).

Group means

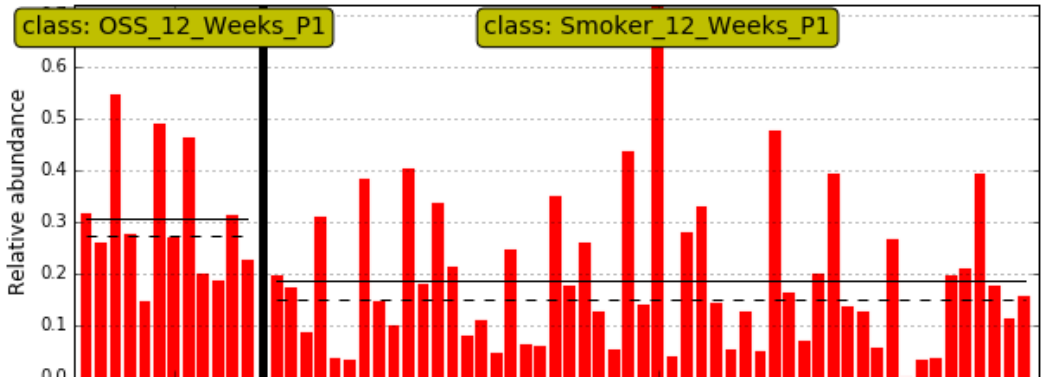
Group medians

LefSe: Health-associated species at 12 weeks: **OSS versus CS**

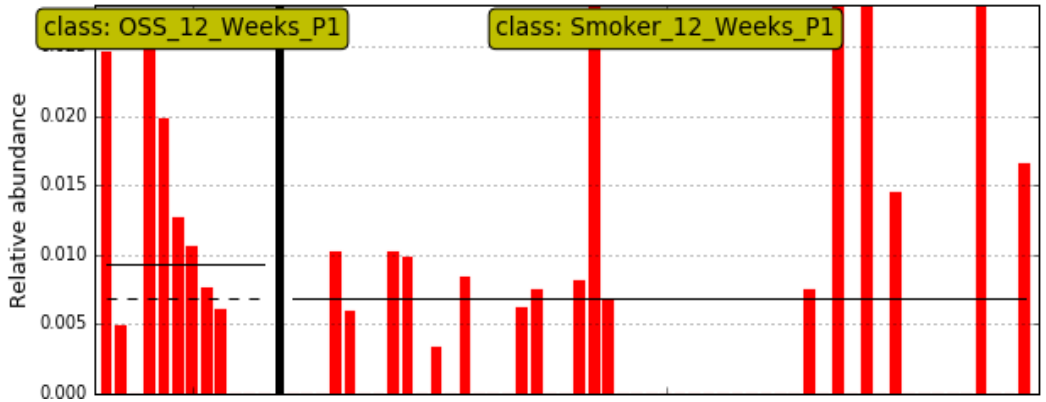
Cornebacterium spp.



Streptococcus spp.



Kingella oralis



- **Health-associated species** were more abundant in NP switchers compared to continued smokers at 12- and 24-weeks (not shown).

Group means

Group medians

Summary and Conclusions

- Switching to NP (OSS) in adult smokers resulted in:
  - more abundant health-associated species (*Corynebacterium, Neisseria, Streptococcus, Rothia, Capnocytophaga spp, Kingella*) at 12 or 24 weeks compared to baseline.
  - reduction in putative pathogens (*Fusobacterium nucleatum,Tannerella, TM7)* at 12 and 24 weeks
  - reduction in some halitosis –associated species (*Solobacterium moorei* and *Fusobacterium spp*)
- With respect to species richness or evenness (Alpha and beta diversity), no significant differences were found at 12 or 24 weeks compared to baseline in any of the groups.
- Minimal or no significant difference was found between DUS vs CS.

Completely switching from cigarette smoking to on!® usage may provide some benefits on the oral microbiome, but the benefit is negated if on!® consumers also smoke.