Hyposalivation but not Sjögren´s Syndrome associated with microbial dysbiosis in women.

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Background

Saliva modulates the environment of the oral biofilm through pH buffer, microbial attachment to host surfaces, and nutritional source. The ecology of stress occurs when a physical factor adversely impacts an ecosystem or its biotic components. Therefore, reduced salivary flow can affect oral-host balance. The leading causes of hyposalivation include disease-associated Sjögren's syndrome (SS) and menopausal women as aging-associated. However, little is known about the oral microbiome integrated with sex hormones in hyposalivation. **The aim of this study was to characterize the hyposalivation microbiome caused by aging or disease affecting the salivary glands in women.**

Methods

We included 50 women older than 40 years of age in any menopausal phase. We collected stimulated saliva from 25 women diagnosed with SS (SS) and 25 without SS (non-SS). Microbial DNA was extracted and amplified by PCR. 16S rRNA gene sequences were used to perform the microbiome analysis with QIIME2 bioinformatic platform. Diversity metrics were estimated, and statistical analysis was carried out. To assess the association between microbial and clinical variables, Pearson Correlation Coefficient was used. For alpha diversity, pairwise Wilcoxon Rank-Sum Test between disease groups and salivary flow in Richness and Shannon indexes was considered. To analyze the differences between the disease and salivary flow groups in Beta diversity, Permutational Multivariate Analysis of Variance was used.
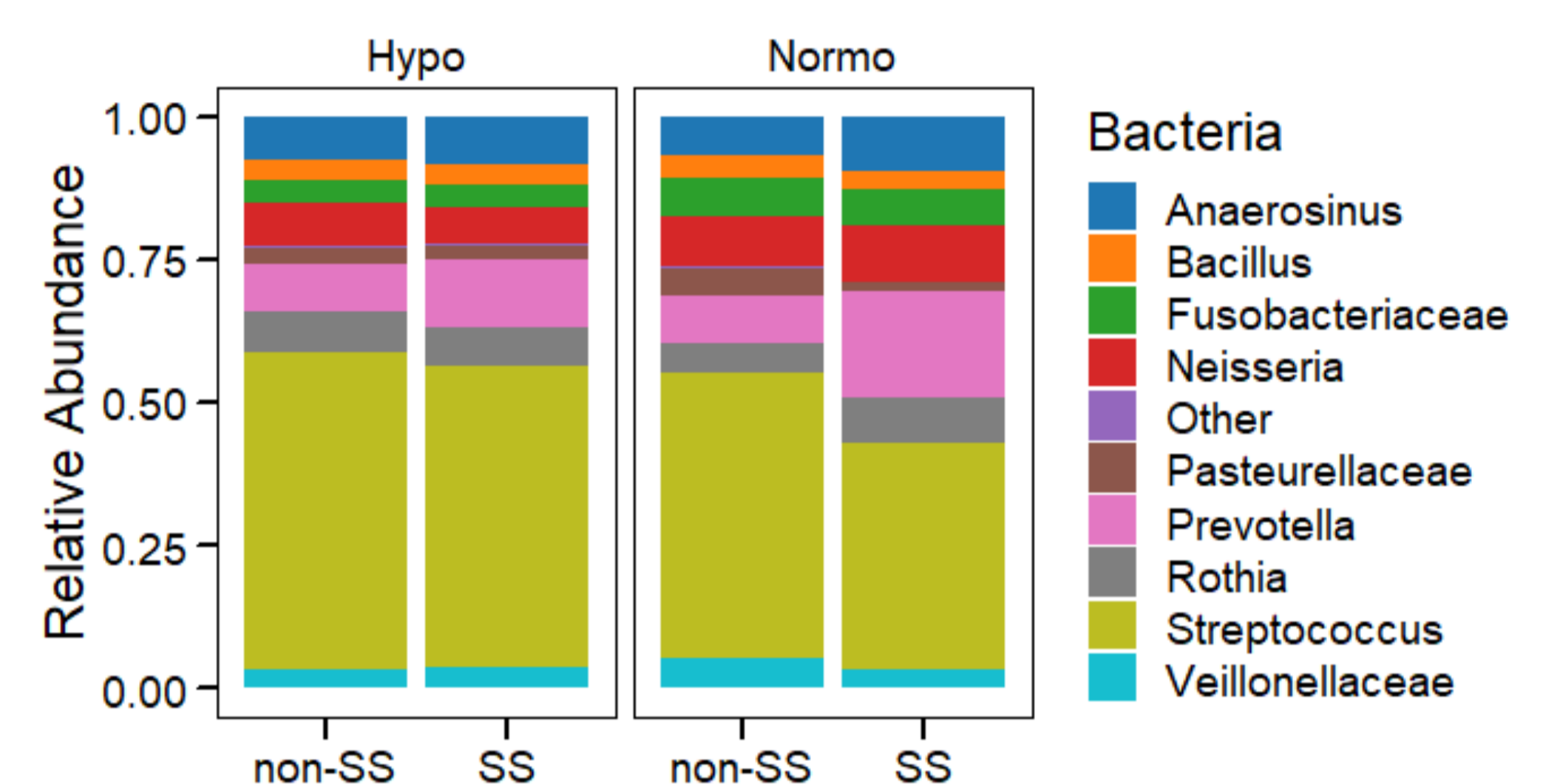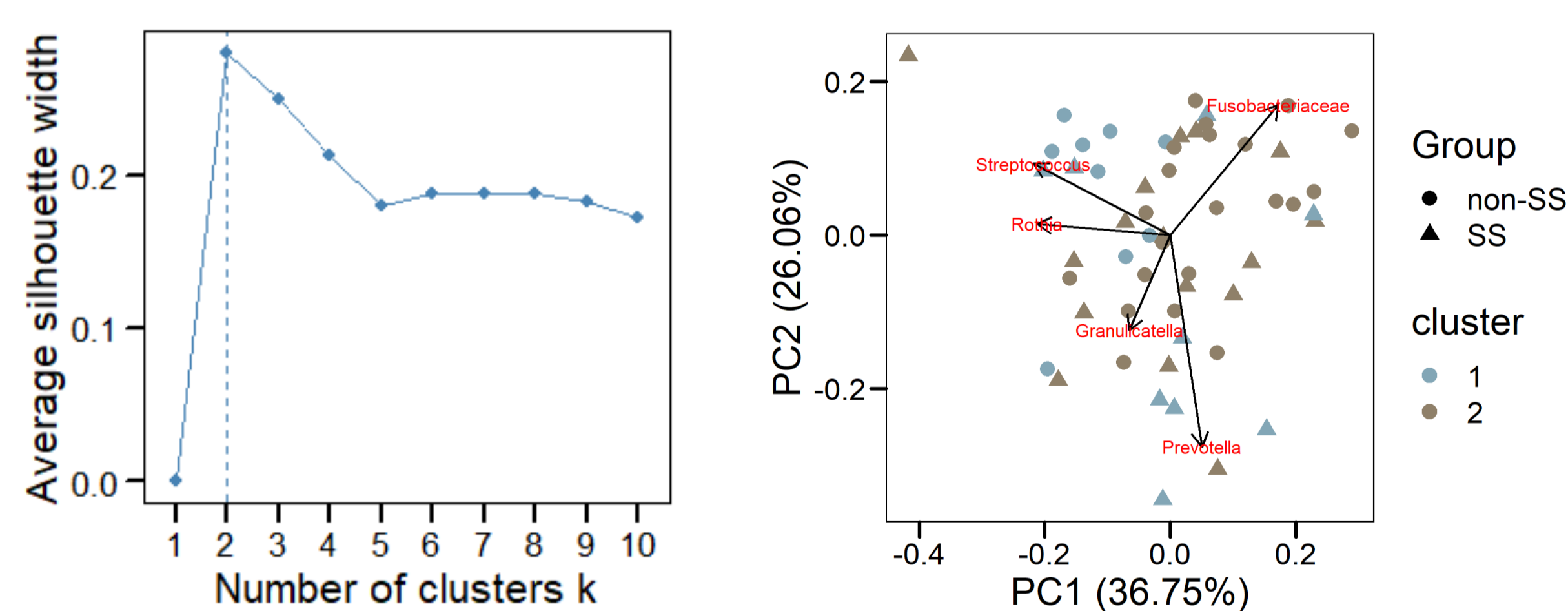
Results

Hyposalivation in women over 40 years
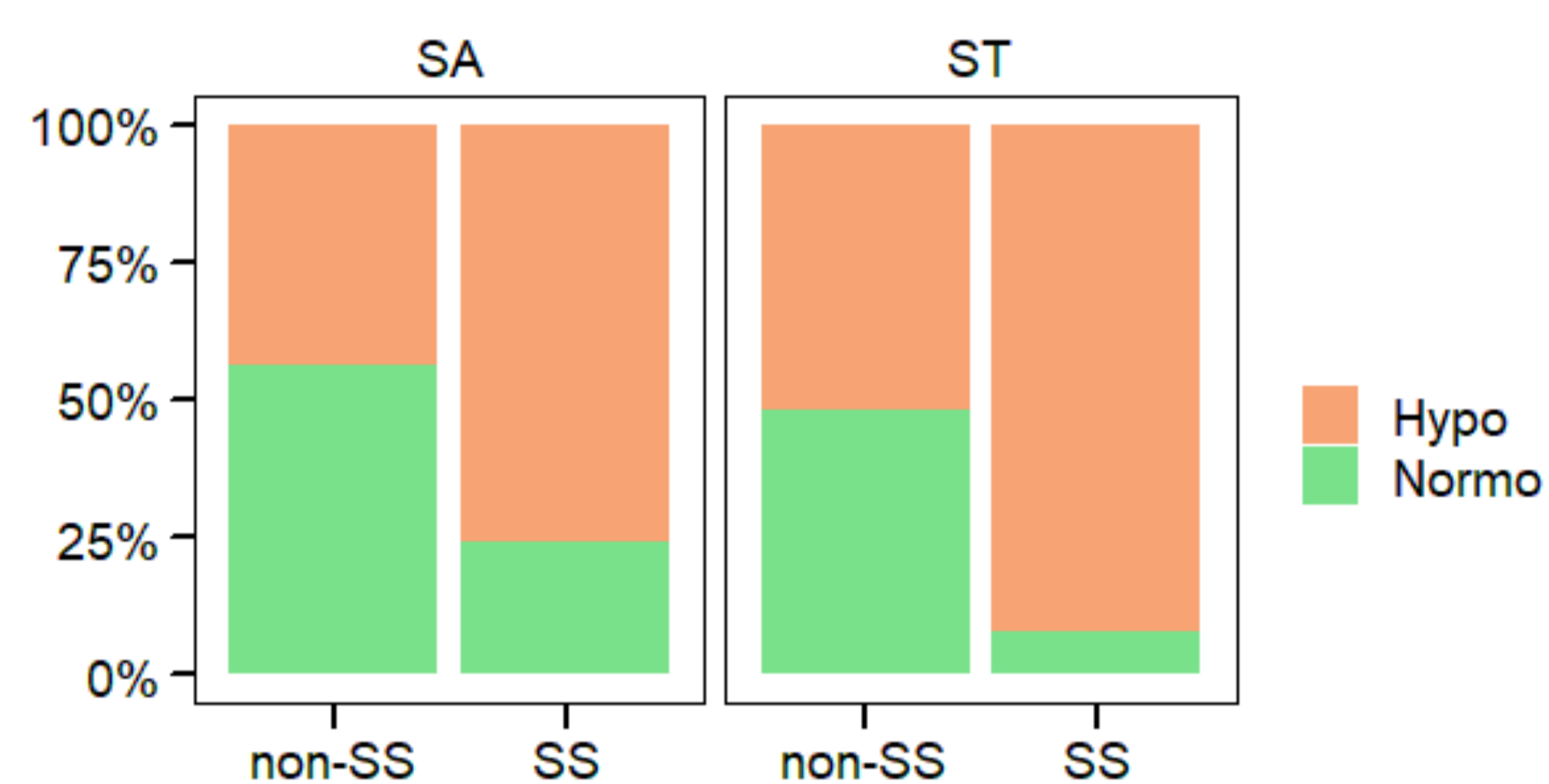
We found an uneven distribution of reduced saliva flow rate in both saliva collected types. In the Sjögren´s Syndrome group the study population has hyposalivation in 76% of the cases in SA and 92% of the cases in SS. Therefore, **SS had accumulative factors of disease and aging that could affect salivary gland tissue and function**, resulting in the reduction of SA and ST.

Streptococcus and *Prevotella* defined microbial clusters

The best number of k-medoids or clusters found in our data was k = 2 determined by unsupervised classification method partition around medoids. We obtained the main taxa separating the dimensions as *Streptococcus*, *Rothia*, *Granullicatella*, *Prevotella*, and *Fusobacteriaceae*. The bacterial genera **Streptococcus** were associated with cluster 1 (P < 0.001) while **Prevotella** was associated with group 2 (P < 0.01).
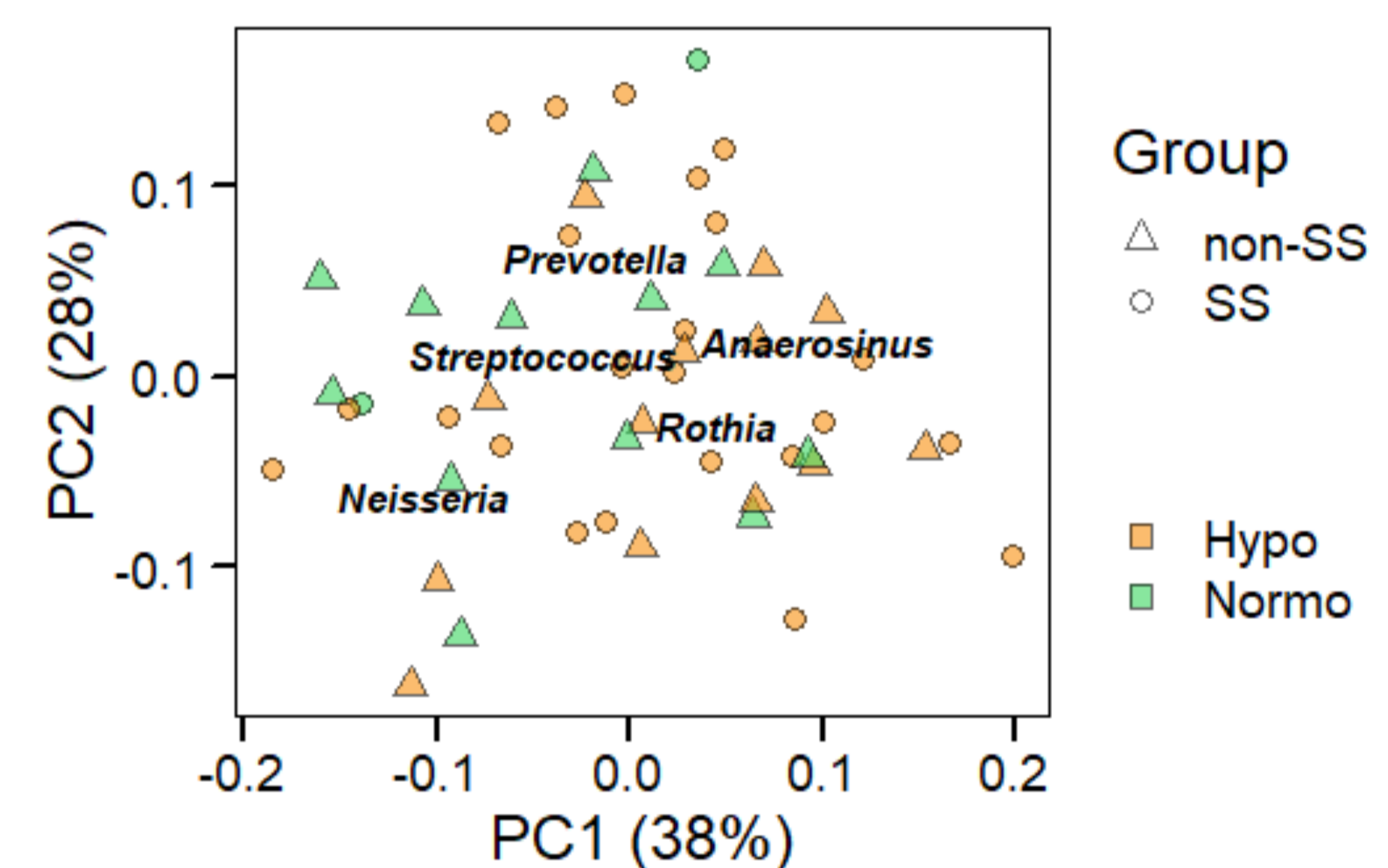
Saliva microbiome in aging and disease

Prevotella, *Neisseria*, *Rothia* and *Streptococcus* are the most abundant taxa with 44% as mean. In women with SS, we found 16% and 10 % for *Prevotella* for normo- and hyposalivation, respectively. In the case of non-SS, the abundance decreases to 8% and 7% for normo- and hyposalivation (figure above). To assess whether the bacterial community was defined by saliva flow or disease in women, we analyzed beta diversity using Weighted Unifrac distances. **We found a microbial community associated with saliva flow, while the diagnosis of Sjögren did not show a significant distribution (P = 0.001 and P = 0.35, respectively, PERMANOVA)** (figure below).

Oral ecological factors driving microbiota distribution
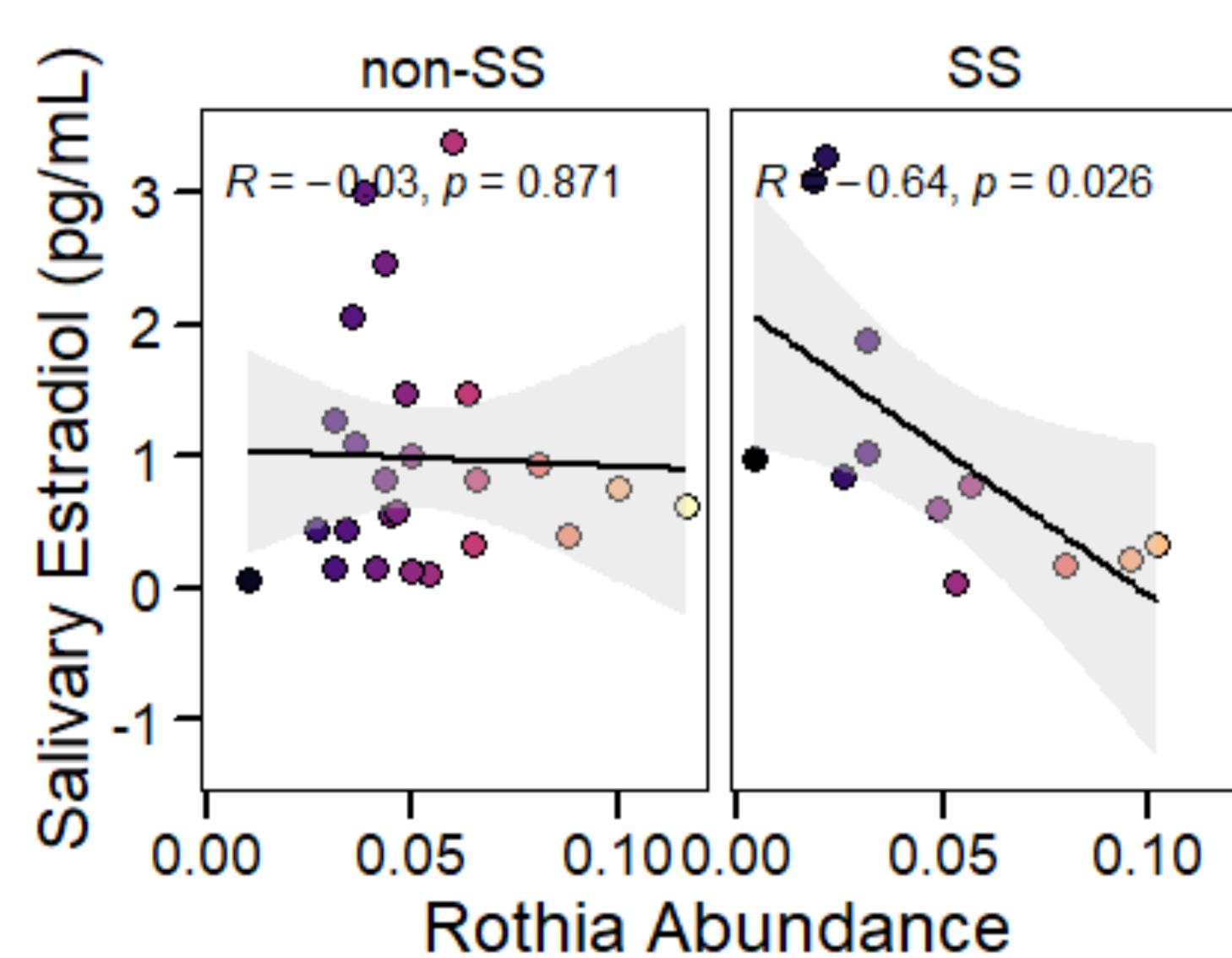
Prevotella decreased with age in the SS group (P = 0.016) but varied independently in the non-SS group (P = 0.58) (right figure). **The genus Rothia was negatively correlated with salivary estradiol only in women affected by Sjögren's syndrome**, suggesting that autoimmune disease could affect oral bacteria (P = 0.026) (left figure). However, *Streptococcus* was not correlated with salivary estradiol for both groups. Overall, we observed that *Prevotella* increased with saliva flow and periodontal status but decreased with age and estradiol levels in women with SS. Interestingly, any of the variables analyzed was not related to non-SS women.
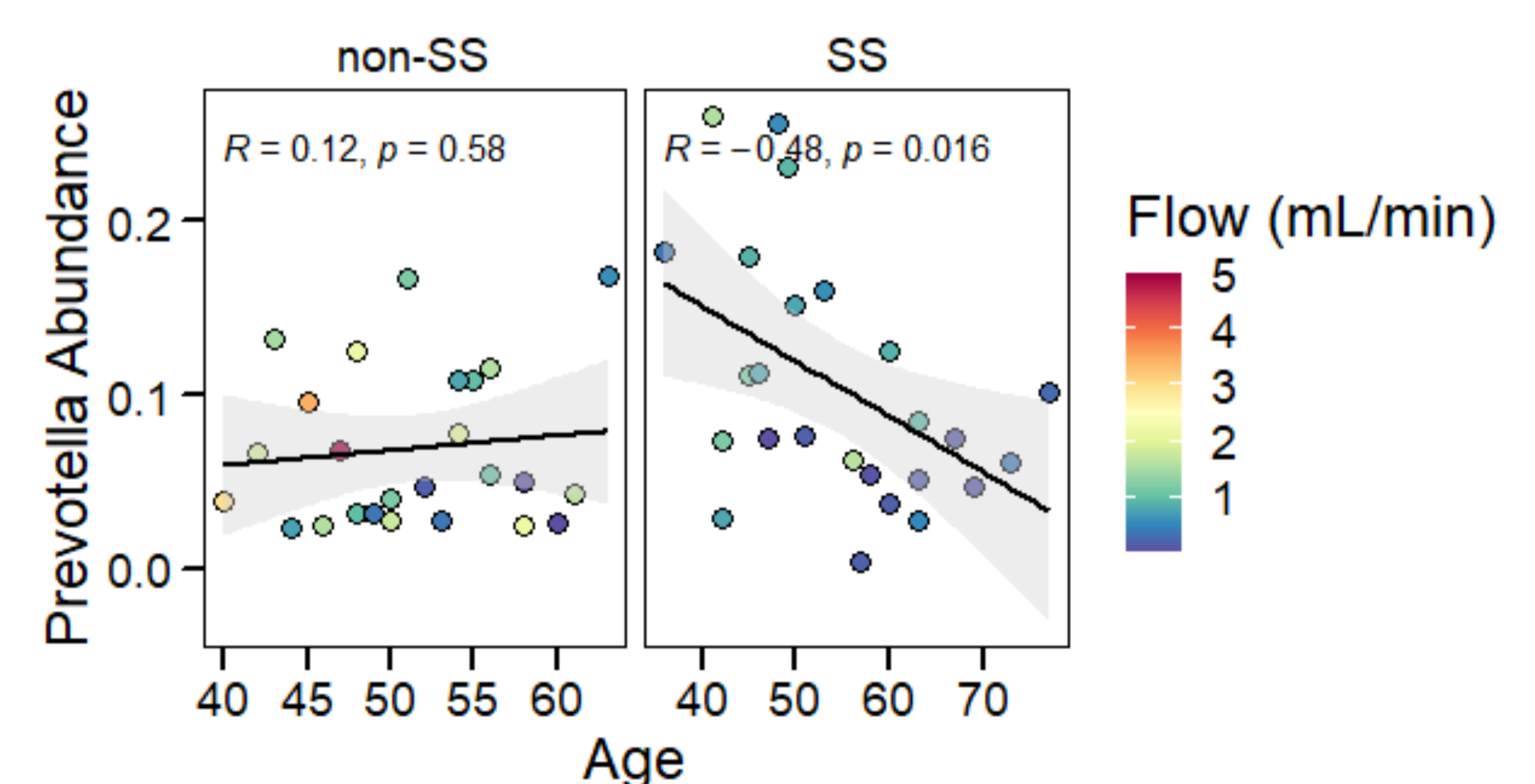
Conclusions

✹ The oral microbiome is not solely determined by SS but rather by the hyposalivation condition.

✹ Two main clusters of microorganisms, *Streptococcus* and *Prevotella* were identified in the hyposalivation microbiome. Thus, intrinsic factors related to the host such as aging, disease, or salivary estradiol might condition the oral ecology and subsequently the salivary microbiome.

✹ *Rothia* and *Prevotella* showed a negative correlation with estradiol and age only in SS women, respectively.

Acknowledgements