

Food Neophobia and scarce olfactory performances are linked to oral microbiota

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ABSTRACT

People suffering from Food Neophobia (FN) tend to follow an unbalanced dietary pattern and show worse olfactory performances. However, scarce data are available on the possible relationships between FN, olfactory performances and the oral microbiota.

The purpose of this work was to understand whether FN and its consequences on orthonasal and retronasal olfaction are related to specific signatures in the oral microbiota. We carried out 16S rRNA gene sequencing of salivary specimens from 83 subjects, whose olfactory performances and Food Neophobia were previously estimated. Our results show that the oral microbiota of people showing high neophobic traits and scarce olfactory performances is enriched in several taxa, such as the periodontal pathogen *Porphyromonas gingivalis*. We hypothesize that these traits are likely attributable to unbalanced dietary patterns, which would need confirmation from dietary records of recruited neophobic subjects.

1. Introduction

Food Neophobia (FN) is defined as the reluctance to eat and/or the avoidance of novel or unfamiliar foods (Pliner & Hobden, 1992). FN is widely recognized as an evolutionary trait since it has protected humans from eating potentially harmful foods for centuries. In addition, it has been proposed to be highly heritable (Knaapila et al., 2007; Faith et al., 2013; Hazley et al., 2022), with the family environment exerting only a marginal effect on this attitude. FN can narrow an individual's food choice (Demattè et al., 2014; Jezewska-Zychowicz et al., 2021), thus limiting dietary variety. Several evidence suggests that high neophobic subjects usually have unhealthier dietary habits compared to neophilics (i.e., those prone to try and accept novel foods), in both childhood (e.g., Perry et al., 2015; Anjos et al., 2021) and adulthood (Knaapila et al., 2015; Sarin et al., 2019). This observation is also supported by a recent study by Jezewska-Zychowicz and colleagues, who explored dietary patterns of neophobic adults, evidencing that they rarely consume

functional foods (e.g., berries, legumes, nuts and superfoods) (Jezewska-Zychowicz et al., 2021).

Another factor strongly influencing human feeding behavior is orthonasal and retronasal olfaction (hereafter "olfaction"). The role of olfaction on shaping food perception and preferences, appetite and dietary habits has been largely explored (Boesveldt, 2017; Morquecho-Campos et al., 2020; Puleo et al., 2021). As an example, an altered sense of smell can influence the intake of salt, sugars or fats, leading to a potentially unbalanced dietary pattern that might result in weight gain or loss, depending on the subjects' adaptive behavior (Kershaw & Mattes, 2018).

Interestingly, olfactory abilities and FN are strongly linked. Indeed, high-neophobics tend to evaluate an odor as less intense and less pleasant than neophilics (Raudenbush et al., 1998), and they show an overall worse olfactory performance than neophilics as measured by the TDI (odor Threshold, Discrimination and Identification) score obtained with the Sniffin' Sticks battery (Hummel et al., 1997; Menghi et al.,

Abbreviations: TDI, Threshold, Discrimination, Identification; FN, Food Neophobia; VOC, Volatile Organic Compound; OTU, Operational Taxonomic Unit.

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2020). Moreover, high-neophobic individuals also exhibited increased anxiety-related physiological responses and a lower extent of retronasal aroma release during the consumption of a strawberry jelly candy. These phenomena might be explained by the tendency of neophobics to avoid unpleasant food-related experiences (Menghi et al., 2020).

The impact of dietary choices on shaping the oral microbiome has been highlighted (De Filippis et al., 2014; Laiola, De Filippis, Vitaglione, & Ercolini, 2020), as well as its potential link to food aroma perception (Piombino et al., 2014). However, information on how Food Neophobia is associated to oral microbiota composition is still lacking. Understanding the relationships existing between food-related personality traits, food choices and oral microbiota is important, since alteration in the microbial composition of the oral cavity potentially caused by unhealthy dietary habits might be related to several oral and systematic diseases and may be indicator of potential food preferences (Lassalle et al., 2018).

In this study, we investigated the microbial composition of salivary samples from subjects whose olfactory performance and Food Neophobia were previously assessed (Menghi et al., 2020) to understand whether these factors, as well as their influence on dietary habits, might result in an alteration of the oral microbiota.

2. Materials and methods

2.1. Study subjects

The present study is part of a larger national scale project aiming to evaluate the prevalence of olfactory impairments in Italy and its associations with biological and cognitive-related covariates (Masala et al., 2019).

Out of the 83 participants involved in our previous investigation (Menghi et al., 2020), 2 participants were excluded due to massive DNA sequencing artifacts. Hence, data from 81 participants (56.7 % females, mean age = 41.5 ± 11.7 yo, mean BMI = 23.92 ± 3.73 Kg/m²; self-reported data) were considered in the present study. More information about participants is provided in a previous report (Menghi et al., 2020). The present study was performed in compliance with the Declaration of Helsinki, and all participants gave their informed and written consent according to the European Data Protection Regulation (UE 679/2016).

2.2. General procedure

Eligible participants were firstly asked to fill out an online questionnaire collecting gender, age, self-reported weight and height, and the Italian version of the FN Scale (IT-FNS; Laureati et al., 2018). FN was calculated by summing up the scores given to the ten items of the IT-FNS, with items ($n = 5$) reflecting neophilic tendencies being oppositely reversed prior the computation (Laureati et al., 2018). To group participants as a function of their FN level, we classified as high-, medium- or low-neophobics those showing pronounced ($n = 15$; IT-FNS score ≥ 36), mild ($n = 32$; $18 < \text{IT-FNS score} < 36$), and low ($n = 34$; IT-FNS score ≤ 18) neophobic tendencies, respectively. These cut-offs were chosen according to a recent study based on a large Italian sample from Laureati and colleagues (Laureati et al., 2018).

Volunteers were then invited to refrain from eating, drinking nothing but water, smoking, and brushing teeth for at least 3 h prior to testing.

During a single 90 min lasting session, volunteers initially provided an unstimulated saliva sample before being comprehensively assessed for olfactory functioning using the standard Sniffin' Sticks battery (Hummel et al., 1997). Olfactory subtests (odor Threshold, Discrimination, Identification) scores were individually calculated as suggested by the Sniffin' Sticks' developers (Hummel et al. 1997), and then added to yield a global score of olfactory functioning (TDI).

For the odor Threshold test, 16 triplets of pens were prepared, with each triplet consisting of two odorless pen and one impregnated with a N-butanol solution. The odorant pen with the highest concentration was

impregnated with a 4% solution of N-butanol, whereas the others were impregnated with progressive 1:2 dilutions. For each triplet, participants were asked to choose which pen was the odorant one. Two consecutive correct answers for the same triplet represented a turning point, which caused the reversal of the staircase, whereas one wrong answer caused the repetition of the task using the step-higher triplet of pens. For each subject, the odor Threshold score was calculated as the geometric mean of the last four turning points out of a total of seven.

For the odor Discrimination test, 16 triplets of pen were prepared, each with two pens with an identical odor, and one different, defined as "target". For each triplet, the participants were asked to identify which pen had the target odor. The odor Discrimination score was computed as the sum of the correct answers.

For the odor Identification test, 16 pens with common odorants were presented to the participants. After smelling each pen, the participants were asked to identify the correct odor from a list of four options. The odor Identification score was computed as the sum of the correct answers.

At the end of the olfactory task, participants observed a 10 min break before being tested for retronasal aroma release by *in vivo* nose-space analysis while consuming, at least in triplicate, a commercial strawberry flavored candy (Fruittella Caramelle Gelee; Perfetti Van Melle; Italy). For this latter purpose, we used a Selected-Ion Flow-Tube Mass Spectrometry (SYFT VOICE 200 ultra, Syft Ltd, New Zealand) to monitor in real time the release of 7 key compounds (i.e., ethyl maltol, 3-hexen-1-ol, ethyl 2-methylbutanoate, (Z)-3-hexenyl acetate, ethyl butanoate, ethyl hexanoate, 2-methylbutanoic acid) exhaled by each participant. Raw spectra from the nose space analysis were pre-treated according to (Charles et al., 2015; Normand et al., 2004) and used to extract the AUC (Area Under the Curve) of the 7 monitored VOCs. More details are provided in our previous paper (Menghi et al., 2020).

2.3. Saliva collection

Volunteers were invited to accurately rinse their mouth with water prior to providing an unstimulated saliva (5 mL) sample by letting it fall into a 50 mL pre-labelled sterile plastic tube (Thermo Fisher Scientific, Waltham, USA) for 5 min. Once collected, saliva samples were centrifuged at 14,000 g for 15 min at 4° C. The supernatant was immediately stored at -80° C until subsequent down-stream applications.

2.4. DNA extraction, PCR amplification and amplicon sequencing

Four mL of saliva were centrifuged at 13,000 g for 2 min. Supernatant was discarded, then DNA was extracted from the cell pellets using the QIAamp® BiOstic® Bacteremia DNA Kit (Qiagen, Hilden, Germany).

The V3-V4 hypervariable region of the 16S rRNA gene was amplified by PCR (about 460 bp), using universal primers S-D-Bact-0341-b-S-17: 5'-CCTACGGGNGGCWGCAG-3' and S-D-Bact-0785-a-A-21: 5'-GAC-TACHVGGGTATCTAATCC-3' (Klindworth et al., 2013). Amplicons were purified and libraries prepared as previously described (Laiola et al., 2020). Briefly, PCR products were purified using AMPure XP beads (Beckman Coulter, Brea, USA), then sequences were barcoded using Nextera XT Indexes (Illumina, San Diego, USA) and pooled in an equimolar pool. Sequencing was carried out on Illumina MiSeq platform, leading to 2x250 bp reads.

2.5. Bioinformatic analysis

Forward and reverse raw reads were joined by FLASH (Magoč & Salzberg, 2011), then sequences were trimmed at the first instance of a base with a PHRED score < 20 , and those that were shorter than 300 bp were discarded using PRINSEQ (Schmieder & Edwards, 2011). The remaining high-quality reads were imported into QIIME 1.9.1 (Caporaso et al., 2010) for following analysis. Briefly, OTUs were de-novo picked at 97% of similarity and representative sequences were mapped against the

Human Oral Microbiome Database (Chen et al., 2010; version 15.1) using the RDP classifier (Wang et al., 2007). OTUs represented by a single sequence were discarded, and samples were rarefied at the same number of reads. Alpha-diversity indices were calculated through QIIME.

2.6. Statistical analysis

Statistical analysis was carried out in a R environment (<https://www.r-project.org>). The function *wilcox.test* (Wilcoxon's rank-sum test) from the *base* package was used to infer statistical differences between the group's medians, unless otherwise stated, choosing a p-value < 0.05 to assess significance.

The function *vegdist* from the *vegan* R package was used to compute pairwise Bray-Curtis distance. The resulting matrix was sent to the command *cmdscale* from the *base* package to produce a PCoA. Only the first two principal coordinates from the PCoA were plotted.

The hierarchical complete-linkage clustering based on Canberra distance was computed and plotted with the function *ph heatmap* (*ph heatmap* R package).

Linear regression between the TDI score and the relative abundance of the taxon were computed through the function *lm* from the *base* package.

Spearman's correlations were calculated using the function *corr.test* from the package *psych* and were plotted through the function *corrplot* (*corrplot* R package). Bubble plots and boxplots were drawn using the functions *geom_boxplot* and *geom_point* (*ggplot2* R package), whereas the upset plot was produced through the function *upset* (*UpSetR* package).

3. Results

3.1. Taxonomic composition of salivary specimens

We identified a core microbiota (i.e., taxa occurring in at least 99% of subjects) at genus level, including 16 taxa (*Actinomyces*, *Rothia*, *Corynebacterium*, *Porphyromonas*, *Prevotella*, *Gemella*, *Granulicatella*, *Streptococcus*, *Lachnoanaerobaculum*, *Veillonella*, *Fusobacterium*, *Leptotrichia*, *Neisseria*, *Campylobacter*, *Haemophilus*, and *Saccharibacteria* (TM7); Table 1). Among these, *Streptococcus* was the most abundant, with a mean relative abundance of 19.7% (± 7.4 %), followed by *Neisseria* (11.4 ± 7.1 %), *Prevotella* (11.0 ± 6.2 %) and *Veillonella* (9.3 ± 4.5 %). These taxa belonged to the phyla of *Firmicutes*, *Proteobacteria*, *Bacteroidetes*. Other abundant genera across all samples were *Porphyromonas* (mean relative abundance of 3.4 ± 2.5 %), *Rothia* (4.9 ± 5.0 %) and *Actinomyces* (4.5 ± 3.2 %).

Table 1

The core microbiota. The table reports: i) the genus, ii) the Phylum, iii) the mean relative abundance of the genus, iv) the relative abundance standard deviation.

| Taxon | Phylum | Mean relative abundance | Std. deviation |
|-------------------------------|------------------------|-------------------------|----------------|
| <i>Streptococcus</i> | <i>Firmicutes</i> | 19.72 | 7.42 |
| <i>Neisseria</i> | <i>Proteobacteria</i> | 11.37 | 7.08 |
| <i>Prevotella</i> | <i>Bacteroidetes</i> | 11.00 | 6.16 |
| <i>Veillonella</i> | <i>Firmicutes</i> | 9.32 | 4.48 |
| <i>Haemophilus</i> | <i>Proteobacteria</i> | 6.36 | 3.53 |
| <i>Rothia</i> | <i>Actinobacteria</i> | 4.89 | 5.00 |
| <i>Actinomyces</i> | <i>Actinobacteria</i> | 4.53 | 3.22 |
| <i>Porphyromonas</i> | <i>Bacteroidetes</i> | 3.42 | 2.47 |
| <i>Fusobacterium</i> | <i>Fusobacteria</i> | 2.94 | 1.60 |
| <i>Granulicatella</i> | <i>Firmicutes</i> | 2.54 | 1.14 |
| <i>Leptotrichia</i> | <i>Fusobacteria</i> | 2.35 | 1.36 |
| <i>Gemella</i> | <i>Firmicutes</i> | 2.23 | 1.55 |
| <i>Saccharibacteria</i> (TM7) | Candidate phylum (TM7) | 1.17 | 1.02 |
| <i>Campylobacter</i> | <i>Proteobacteria</i> | 0.67 | 0.58 |
| <i>Corynebacterium</i> | <i>Actinobacteria</i> | 0.60 | 0.66 |
| <i>Lachnoanaerobaculum</i> | <i>Firmicutes</i> | 0.43 | 0.33 |

In order to investigate the ecological relationship between microbial taxa, we looked at specific co-occurrence and co-exclusion patterns (Fig. 1). Co-occurrence analysis highlighted the presence of 2 distinct and co-excluding groups of taxa (Fig. 1). More specifically, the first group included the genera *Actinomyces*, *Atopobium*, *Veillonella*, *Prevotella*, *Megasphaera* and *Selenomonas*, whereas the other included *Porphyromonas*, *Aggregatibacter*, *Neisseria*, *Capnocytophaga*, *Fusobacterium*, *Gemella* and *Haemophilus*.

3.2. Hyposmic subjects show specific traits in their salivary microbiota and higher abundance of potentially pathogenic species

As proposed by Oleszkiewicz et al. (2019), a TDI value of 30.75 can differentiate subjects with overall scarce olfactory performances (i.e., "hyposmic") from people with normal olfactory performances (i.e., "normosmic"). According to this cutoff, 7 out of 81 subjects involved in this study were labeled as "hyposmic". Principal Coordinate Analyses (PCoAs) based on Bray-Curtis distance performed at any taxonomic level did not properly separate hyposmics from normosmics, suggesting that the microbial community structure was not influenced by the TDI score. In addition, none of the alpha-diversity scores showed a significant difference between the two groups.

However, we observed significantly higher abundance of *Granulicatella*, *Parvimonas* and *Peptidiphaga* in hyposmics ($p < 0.05$), while *Pseudopropionibacterium* showed a higher relative abundance in normosmics ($p < 0.05$). Since it has been previously observed that TDI is negatively correlated with BMI in the same cohort (Menghi et al., 2020), we checked if a correlation existed between differentially abundant taxa and BMI. However, none of the previously reported genera were significantly correlated with BMI, thus excluding its influence on the observations.

We also stratified the subjects into quartiles based on TDI score, in order to compare the salivary microbiota between subjects with the highest and lowest values of TDI. In particular, group 1 included 21 subjects, with a TDI score ranging between 19.25 and 33 (1st quartile), while group 2 included 20 subjects, with a TDI score ranging between 38.5 and 44 (4th quartile; Fig. 2a).

At species level, *Porphyromonas gingivalis*, *Capnocytophaga granulosa*, *Fusobacterium periodonticum* and *Aggregatibacter* sp. were significantly more abundant in subjects belonging to group 1 ($p < 0.05$), together with an unassigned species belonging to the genus *Kingella* ($p = 0.01$). On the other hand, group 2 showed a higher relative abundance of *Selenomonas noxia* ($p = 0.02$) and *Actinomyces gerencseriae* ($p = 0.02$) (Fig. 2b). Hierarchical complete-linkage clustering based on the relative abundance of these species (Fig. 3) effectively separated group 1 (i.e., low TDI group) and 2 (i.e., high TDI group). Again, none of these taxa was statistically correlated with BMI.

In addition, we observed that *Porphyromonas gingivalis* and *Filifactor alocis* showed a strong negative correlation with the TDI score (adjusted $R^2 = 0.039$, $p = 0.037$; adjusted $R^2 = 0.0459$, $p = 0.031$; Fig. 4).

Interestingly, *Porphyromonas catoniae* and *Fusobacterium nucleatum* subsp. *vincentii* were negatively correlated with the TDI score (adjusted $R^2 = 0.042$, $p = 0.03$; adjusted $R^2 = 0.055$, $p = 0.02$), while no correlation was observed with *Fusobacterium nucleatum* subsp. *animalis* (Fig. 4). On the contrary, *Prevotella* sp. HMT 309 (adjusted $R^2 = 0.066$, $p = 0.009$) and *Slackia exigua* (adjusted $R^2 = 0.089$, $p = 0.004$) were positively correlated with the TDI score (Fig. 4).

3.3. Effect of Food Neophobia on oral microbiota

Menghi and colleagues previously observed that higher levels of neophobia were associated with worse global olfactory performances on the same cohort (Menghi et al., 2020). Here, we tested whether the salivary microbial community structure or the abundance of specific taxa changed significantly between high-, medium- or low-neophobics and found that the FN groups were not characterized by overall

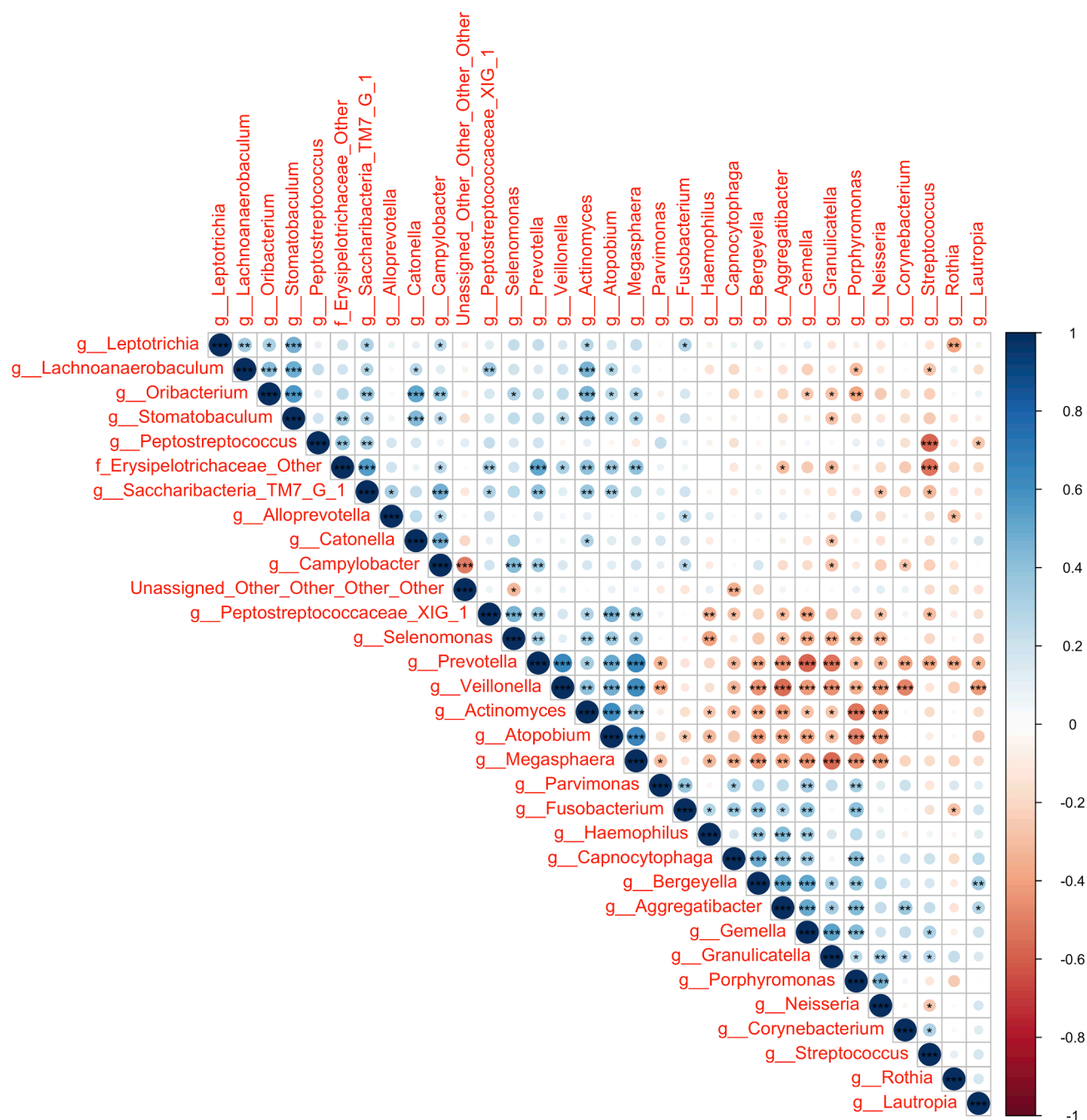


Fig. 1. Correlation matrix at genera level based on Spearman's rank correlation coefficient. Only taxa present in at least 90% of subjects are shown. *, p-value \leq 0.05; **, p-value \leq 0.01, ***, p-value \leq 0.001.

changes in the microbial community, as shown by a PCoA based on the Bray-Curtis distance (Supplementary Fig. 1). Moreover, the FN score was slightly higher in hyposmics, even though the result was not significant, probably due to the small sample size.

Interestingly, some differences were observed at single taxon level. For instance, *Alloprevotella* sp. HMT 473 and a species belonging to cluster XI of the family *Peptostreptococcaceae* were significantly more abundant in low-neophobic subjects (Kruskal-Wallis test, $p < 0.05$), whereas *Klebsiella pneumoniae* and *Scardovia wiggisiae* prevailed in high-neophobics.

3.4. Correlation analysis between microbiota composition, FN, retronasal aroma release and olfactory scores

A correlation analysis between microbial species abundances, FN, VOCs (Volatile Organic Compounds, Menghi et al., 2020) release in the retronasal space and both single (Threshold, Discrimination, Identification) and cumulative (TDI) olfactory scores was carried out. Only taxa showing at least 2 significant correlations according to Spearman's correlation index were reported. We observed that the genera *Bifidobacterium*, *Bacteroides*, *Mitsuokella* and *Klebsiella* were negatively correlated to the release of VOCs in the retronasal space. Of these, *Bifidobacterium* showed the highest number of negative correlations with VOCs release (5 out of 7), while *Klebsiella* was the only taxon also showing a positive association with the FN score (Supplementary Fig. 2).

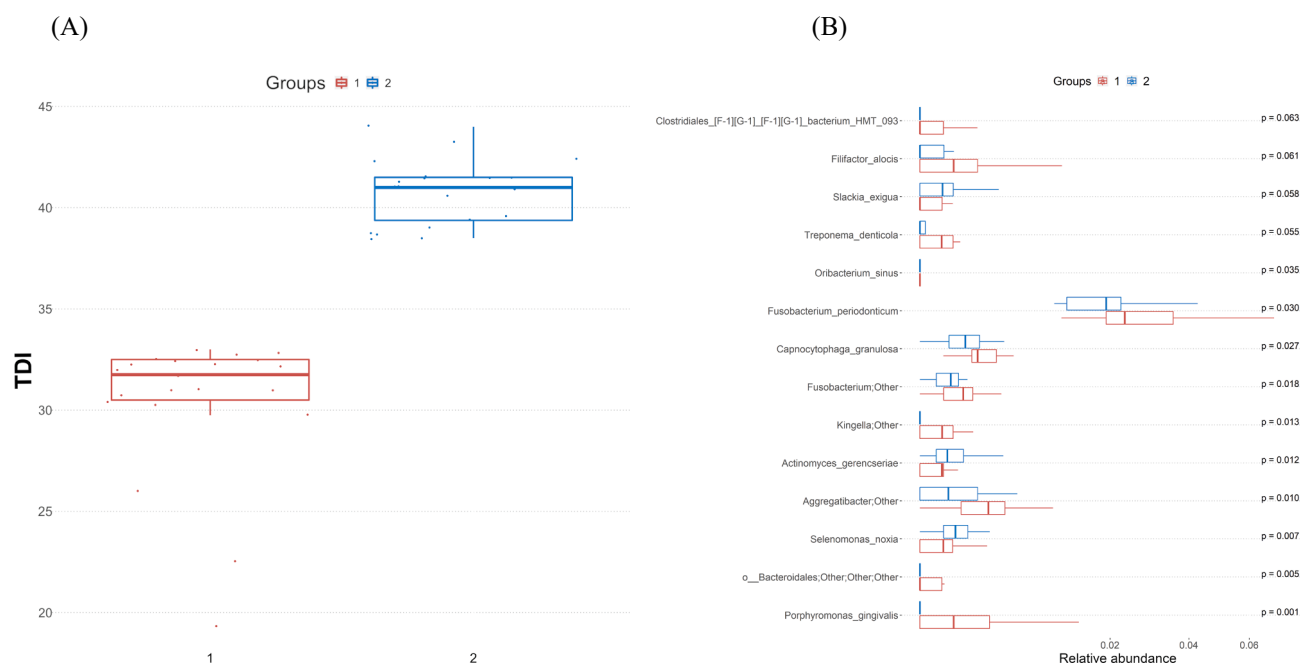


Fig. 2. A) TDI quartiles. Quartile 1 corresponds to group 1, whereas quartile 4 corresponds to group 2. B) Boxplots showing the relative abundance of significantly different species between group 1 and group 2.

Interestingly, *Porphyromonas* and *Fusobacterium* had a negative association with Threshold and Discrimination scores, as well as with the cumulative TDI score, whereas *Slackia* was positively correlated with Identification and TDI. These results were consistent with those observed from the regression analysis.

In addition, subjects were stratified into quartiles based on each of the monitored VOCs, then the abundance of taxa was compared between the extreme quartiles obtained from each VOC. As a result, we observed that *Streptococcus parasanguinis* clade 411 was the only species showing a significant difference between extreme quartiles for 4 VOCs (i.e., ethyl 2-methylbutanoate, ethyl hexanoate, 3-hexen-1-ol and ethyl butanoate), always resulting more abundant in groups with a lower volatile release (Supplementary Fig. 3).

4. Discussion

The salivary microbial composition from the participants was largely consistent with those reported in previously published studies, which identified a similar “core” microbiota (Bik et al., 2010; Takeshita et al., 2016; Laiola et al., 2020). Indeed, as reported in literature, we observed a great inter-individual variability.

Focusing on differences between people with different olfactory performances, we observed that subjects with a lower TDI score (i.e., those belonging to group 1) showed a significantly higher abundance of *Porphyromonas gingivalis*, a microorganism belonging to the “red complex” (Socransky et al., 1998), which is associated with periodontal disease progression (Mohanty et al., 2019). In addition, *Fusobacterium nucleatum* subsp. *vincentii* and *Filifactor alocis* showed a negative correlation with the TDI score. The former belongs to a species which is able to co-aggregate with early and late subgingival colonizer, thus enhancing the dental biofilm formation (Mohanty et al., 2019), whereas the latter has been recently suggested as a putative oral pathogen (Vashishta et al., 2019).

On the other hand, our results showed that higher values of TDI are associated with taxa normally considered as markers of eubiosis, such as *Actinomyces* spp. For instance, the genus *Actinomyces* was reported as discriminant for caries-free subjects (Eriksson et al., 2017), while *A. gerencerisae* was more abundant in healthy control than in patients with

aggressive periodontal disease in a previous report (Gonçalves et al., 2012). However, studies reporting the influence of *Selenomonas noxia* on the oral health are contrasting, since several authors reported the species as an oral health marker (Zhou et al., 2013; Panda et al., 2020), whereas others evidenced its potential role in caries formation (Eriksson et al., 2017) and periodontitis initiation (Tanner, 2015). These results indicate that oral health can be linked to olfactory performances.

In accordance with a previous study on nasal microbiota in people with olfactory dysfunction (Koskinen et al., 2018), we highlighted that the genus *Porphyromonas* was more abundant also in the oral microbiota of people with reduced TDI. Moreover, it is worth noting that the taxa that were enriched in the low-TDI group (e.g., *Porphyromonas gingivalis* and *Treponema denticola*) have been also reported as enriched in subjects consuming an unhealthy diet. For instance, Tennert and colleagues observed the effects of a diet rich in fruit, vegetables and fibre, and low in highly processed sugars on the dental plaque and the salivary microbiota, highlighting a significant decrease of *Granulicatella* spp. and *Fusobacterium* spp. (Tennert et al., 2020). In the same vein, Laiola et al. reported a significant reduction of *P. gingivalis* and *T. denticola* in the salivary microbiota of obese and overweight subjects after a 8-week Mediterranean diet-based intervention. These species were significantly and negatively correlated with fiber intake, thus opening new scenarios about the metabolism of periodontopathogenic species (Laiola et al., 2020).

Mediterranean diet (MD) is widely recognized as a healthy dietary pattern (De Filippis et al., 2016; Ventriglio et al., 2020), which provides several antioxidants and anti-inflammatory compounds (Mentella, Scaldaferrri, Ricci, Gasbarrini, & Miggiano, 2019; Meslier et al., 2020). Interestingly, a recent study investigated the relationship between FN and adherence to the MD, showing that neophobic subjects had a significantly lower adherence to the MD, and highlighting how they are likely to assume a lower intake of potentially beneficial foods (Predieri et al., 2020).

In addition, although the relative abundance of some taxa such as *T. denticola* and *P. gingivalis* was generally low in our cohort (i.e., mean relative abundance < 1%), evidence in the literature suggests that even low magnitude shifts in the abundance of oral pathogens might exert a significant effect on the community structure (Hajishengallis et al.,

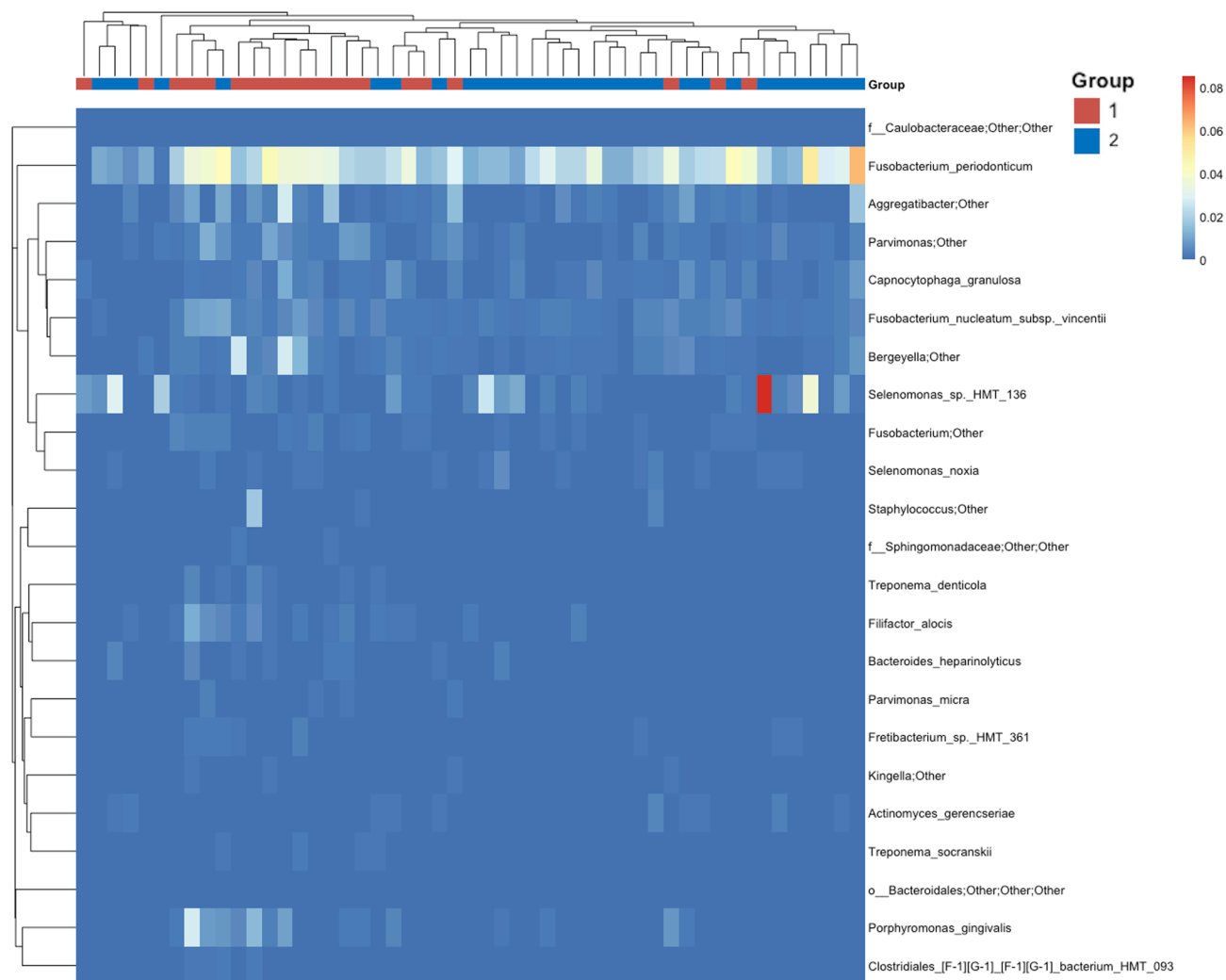


Fig. 3. Hierarchical complete-linkage clustering of subjects belonging to groups 1 and 2 based on discriminant species. Canberra metric was used to compute pairwise distances. The column bar colors denote the membership of subjects to the groups.

2011). In this sense, the pathogenesis of *P. gingivalis* is emblematic: all the strains of this species produce a protease called gingipain, which not only exerts a cytopathic activity, but also acts as a ligand between *P. gingivalis* and other pathogenic microorganisms (Jia et al., 2019), thus fostering their growth. Since *P. gingivalis* can exert a pathogenic activity regardless of its abundance in the community, it has been termed a “keystone pathogen” (Hajishengallis et al., 2011; Olsen et al., 2017). The results from the co-occurrence analysis corroborate this idea: the genus *Porphyromonas* is significantly associated with other potential pathogenic taxa, such as *Capnocytophaga* and *Fusobacterium*, whereas it is anticorrelated with eubiosis-related taxa (e.g., *Prevotella*, *Veillonella* and *Actinomyces*; Fig. 1).

Although the overall oral microbiota composition was not different between high-, medium- and low-neophobics, some differences were observed at single taxon level, highlighting that FN may be linked with the presence of potential pathogenic and caries-associated species. High-neophobic subjects showed higher abundance of *Scardovia wiggsiae*. This species, which belongs to the family of *Bifidobacteriaceae*, has recently been reported to be aciduric and acid producer (Kameda et al., 2020), thus strongly associated with caries formation in both infants (Matondkar et al., 2020) and adolescents (Kressler et al., 2017; Eriksson et al., 2017).

In addition, *Klebsiella pneumoniae* was enriched in high-neophobics. This species is recognized as a biofilm producer in the oral cavity (Leoney et al., 2020), as well as an antibiotic resistant opportunistic

pathogen, often involved in several infections including, but not limited to, pneumonia (Paczosa & Mecsas, 2016). Notably, several researchers showed that strains of *Klebsiella pneumoniae* are able to produce biofilm in acid environments (Nicolau Korres et al., 2013), similarly to *S. wiggsiae*. Although oral biofilms can host a wide range of homeostatic microorganisms, the development of dental plaque in an acid environment is associated with several disease-related species (Marsh, 2009).

Among the factors potentially influencing food choice, retronasal smell is one of the most important (Ployon et al., 2017). Menghi et al. (2020) observed a significantly lower extent of VOCs released by high-neophobic subjects than neophobics, which was associated with a hostile arousal response and with an anxious behavior towards food. However, the volume of VOCs reaching the receptors is not only dependent on oral processing, but also on physiochemical properties of the food matrix and on its interactions with salivary compounds (Frank et al., 2015; Tarrega et al., 2019). For instance, a high concentration of proteins in saliva might lead to volatile-protein interactions that in turn limit the perception of retronasal aroma, as we previously observed (Piombino et al., 2014) in obese subjects. In addition, planktonic cells suspended in saliva may influence the release of aroma compounds from food matrices, as it has been demonstrated *in vitro* (Ly et al., 2008) and *ex vivo* (Muñoz-González et al., 2015). In fact, oral bacteria are the major source of salivary glycosidase enzymes (Parker et al., 2020), which are in turn necessary to release several VOCs from glycosylated precursors. Since the ratio between glycosylated and free volatiles is high in

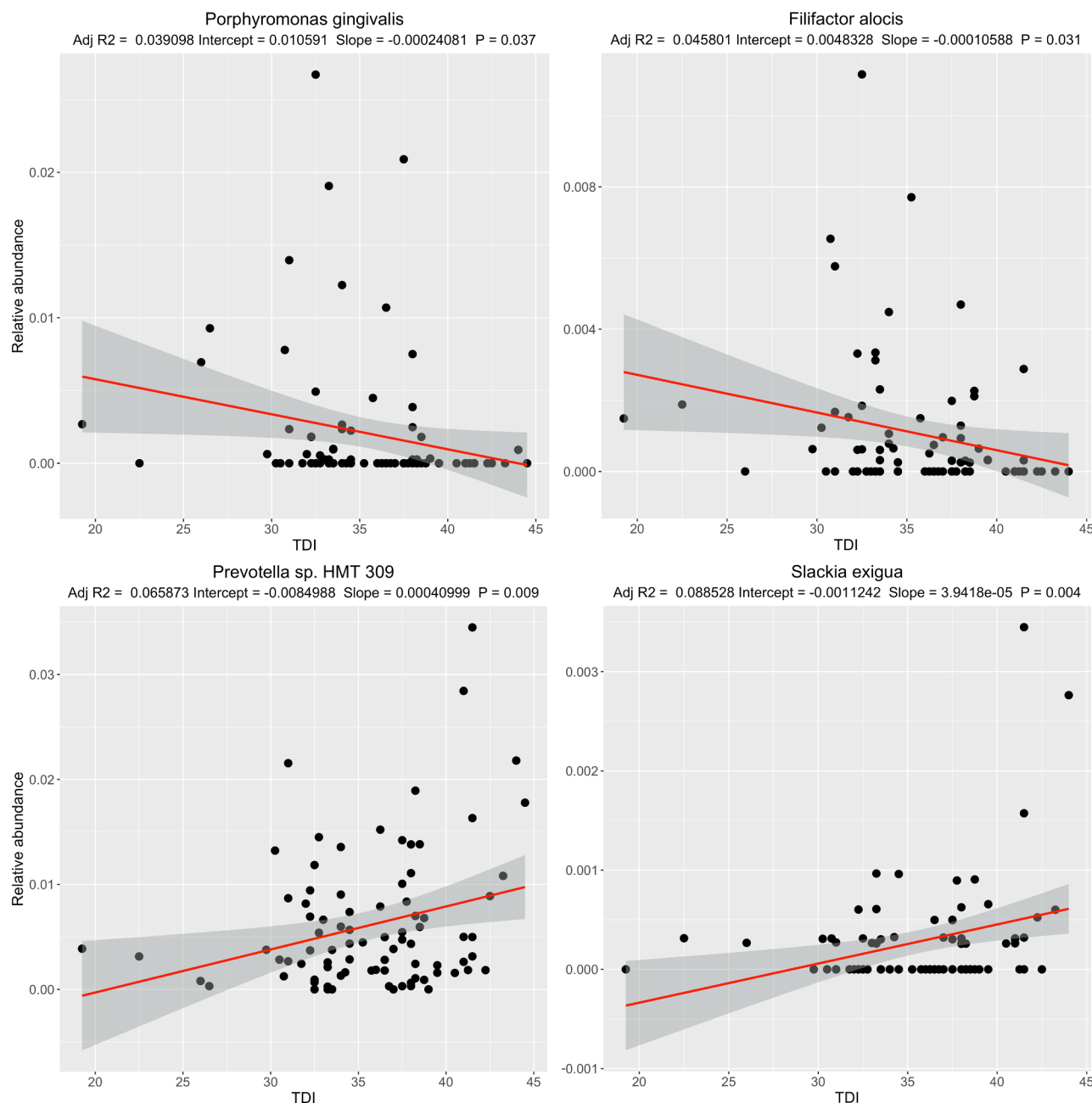


Fig. 4. Significant linear regressions between oral taxa and the cumulative TDI score.

strawberries for industrial processing (e.g., strawberries used as ingredients for candies) (Gaborieau et al., 2018), we explored our data in order to assess whether the release of VOCs is linked not only with the consumer's behaviour (as previously demonstrated for this cohort by Menghi and colleagues), but also with the abundance of specific microbial taxa in the oral microbiota.

From the correlation matrix, *Bifidobacterium* spp. reported the highest number of significant ($p < 0.05$) correlations with the total amount of VOCs released during the experiments (expressed as AUC, Area Under the Curve), followed by the genus *Peptostreptococcus* (Supplementary Fig. 2). Moreover, *Streptococcus parasanguinis* clade 411 was more abundant in the oral cavity of people with a minor release of 4 out of 7 volatiles during mastication. This species has been recently advised as associated with a high grade of dental pathology (Relvas et al., 2021) and with smokers' oral microbiome (Sato et al., 2020). However, mechanisms underlying these observations are unclear, and a further

investigation is needed in order to clarify the metabolic contribution of each taxon to the aroma release.

To the best of our knowledge, this is the first study that attempts to investigate the influence of FN and olfaction on the oral microbiota. Taken collectively, our results suggest the existence of a link between dietary habits, olfactory performances and the composition of the salivary microbiota. Indeed, we hypothesize that FN and scarce olfactory performances might influence the food choice, leading to an alteration of the salivary microbiota composition through the selection of several oral pathogenic taxa.

5. Conclusion

As previously proposed by Menghi and colleagues, FN has a considerable impact on both olfactory performances and VOCs release during mastication, thus probably shaping food choice and indirectly

influencing the salivary microbiota composition. We observed that high levels of neophobia and the inclination to perceive odors at a lower extent are associated with a higher abundance of several dysbiosis-related taxa in the salivary microbiota. Since it has been previously observed that high-neophobics show a lower adherence to MD and thus they are likely to follow an imbalanced dietary pattern, we suggest that the influence of FN and arousal toward food on feeding behavior might jeopardize the oral microbiome.

Unfortunately, the lack of detailed dietary records is limiting the confirmation of such hypothesis.

In addition, due to the limited sample size and the unbalanced distribution of hyposmics and normosmics, some of the observations might not be representative of the entire population.

However, to the best of our knowledge, the relationship that occurs between FN, olfaction and oral microbiota have not been explored previously. Therefore, further investigation involving a larger cohort and integrated with more detailed information about subjects' FN, olfactory performances and dietary habits might use our result as a starting point, to better explain the influence of these factors on the composition and on the metabolism of the oral microbiome.

CRedit authorship contribution statement

Vincenzo Valentino: Investigation, Formal analysis, Writing – original draft, Visualization. **Francesca De Filippis:** Conceptualization, Supervision, Writing – review & editing. **Leonardo Menghi:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Flavia Gasperi:** Conceptualization, Writing – review & editing. **Danilo Ercolini:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodres.2022.111092>.

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