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Rapid shift of gut microbiome and enrichment of beneficial microbes during arhatic yoga meditation retreat in a single-arm pilot study

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Abstract

Background The human microbiome plays a vital role in human health, mediated by the gut–brain axis, with a large diversity of functions and physiological benefits. The dynamics and mechanisms of meditations on oral and gut microbiome modulations are not well understood. This study investigates the short-term modulations of the gut and oral microbiome during an Arhatic Yoga meditation retreat as well as on the role of microbiome in improving well-being through a possible gut-brain axis.

Methods A single-arm pilot clinical trial was conducted in a controlled environment during a 9-day intensive retreat of Arhatic Yoga meditation practices with vegetarian diet. Oral and fecal samples of 24 practitioners were collected at the start (Day0: T1), middle (Day3: T2), and end (Day9:T3) of the retreat. Targeted 16S rRNA gene amplicon sequencing was performed for both oral and gut samples. Functional pathway predictions was identified using phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2). DESeq2 was used to identify the differential abundant taxa. Various statistical analyses were performed to assess the significant changes in the data.

Results Our findings revealed that Arhatic Yoga meditation together with a vegetarian diet led to changes in the oral and gut microbiome profiles within the 9-day retreat. Oral microbiome profile showed a significant ($p < 0.05$) difference in the species richness and evenness at the end of study, while non-metric multidimensional scaling (NMDS) confirmed the shift in the gut microbiome profile of the practitioners by T2 timepoint, which was further supported by PERMANOVA analysis ($p < 0.05$). Health-benefiting microbes known to improve the gastrointestinal and gut-barrier functions, immune modulation, and gut-brain axis were enriched. Gut microbiome of both beginner and advanced Arhatic Yoga practitioners showed similar trends of convergence by the end of study. This implies a strong selection pressure by Arhatic Yoga meditation together with a vegetarian diet on the beneficial gut microbiome.

Conclusion This pilot study demonstrates that Arhatic Yoga meditation practices combined with a vegetarian diet during a short intensive retreat resulted in enrichment of known health-promoting microbes. Such microbial

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consortia may be developed for potential health benefits and used as probiotics to improve the gastrointestinal and immune systems, as well as functions mediated by the gut-brain axis.

Trial registration Study was submitted in <https://clinicaltrials.gov/on28-02-2024>. Retrospective registered.

Keywords Arhatic yoga, Oral and gut microbiome, Beneficial microbes, Clinical trial, Gut-brain axis, Meditation

Introduction

The gut microbiome is a complex ecosystem of trillions of microorganisms residing in the gastrointestinal tract [1]. Rising number of studies have pointed out that the gut microbiome has a significant influence not only on gastro-intestinal health but on the development and progression of systemic diseases such as cardio-metabolic, auto-immune, and neuropsychiatric [2–6]. The stability of human gut microbiome varies with host genetics, age, lifestyle, and diet [7]. In addition, physical activity, exercise, and yoga changes the gut and oral microbial composition with possible benefits for the host [8–10].

The gut microbiome influences the maintenance of health and disease development and progression through the production of metabolites and signalling molecules which then enter systemic circulation and have an effect on the metabolic, immunologic, and homeostatic functions of different organs [11–13]. As such a balanced gut microbiome (eubiosis) facilitates the right composition of bacteria derived metabolites to maintain human health and prevents disease development and progression. On the contrary an imbalanced gut microbiome (dysbiosis) can lead to increased oxidative stress, inflammation, and gut-barrier permeability as well as metabolic dysfunction [14, 15]. This in turn, will have detrimental effects on the pathophysiology and subsequent development and progression of disease.

Given the above, it is not surprising that there has been a rising focus on the evaluation of therapies and interventions that promote gut eubiosis. Plant-based diet, supplementation with pre- and probiotics and gut faecal transplantation are well known to modulate the gut microbiome [16–18]. Additionally, few studies have demonstrated that active components of the plant secondary metabolites and/or plant extract improve lipid metabolism, enhance intestinal gut barrier function, inhibit fat accumulation, reduce inflammation, and regulate the abundance of gut microbiome, which ultimately improve the health state of the host [19–23]. Meditation practices, exercise, proper diet, and yoga are increasingly being incorporated as therapeutic interventions to improve mental and health disorders and have been suggested in several studies to have a beneficial effect on gut microbiota composition [9, 24–28]. However, the understanding of the role of meditation in gut and oral microbiome modulation is not well understood. A systematic review

highlighted the importance of meditation in regulating the stress response and promoting the healthy gut-barrier function [25]. This review also recommended that it is necessary to explore the effects of meditation on gut microbiome and the functions in oral-gut axis. Moreover, several reports suggest that psychological stress may also affect the oral microbiome along with alteration in gut microbiota through oral-brain axis and oral-gut axis, respectively [29–31]. Direct link between gut homeostasis and stress-related disorders have also been reviewed recently [32, 33]. Upon stress, hypothalamus releases corticotropin-releasing factor (CRF) which disrupts the gastrointestinal system including oxidative damage to the colon, inflammation to the intestine, dysfunction intestinal barrier and triggers immune response [33–37]. Hence, it is intriguing to understand the role of Arhatic Yoga practices, which includes a vegetarian diet, in modulation of both oral and gut microbiome.

The present study was designed as a single-arm pilot scale to understand the temporal changes in both gut and oral microbiome of the practitioners at a meditation-cum-spiritual retreat in a controlled environment. Here, the study and its outcomes are reported according to Biofield Therapies: Reporting Evidence Guidelines (BiFi REGs) recommendations [38]. In brief, an Arhatic Yoga spiritual retreat was conducted for 9 days in Bovina, New York, USA. Arhatic Yoga is an advanced spiritual practice to enhance the physical, emotional, and mental aspects of the practitioner through an intensive daily regimen of physical and breathing exercises, meditations together with vegetarian diet, energetic purifications, self-awareness practices and spiritual studies. The energetic purifications are based on the hands-off modality of Pranic Healing, where the practitioners typically stand 50–60 cm away from the subjects [39]. This study provides a unique opportunity to test the hypotheses that (1) Arhatic Yoga practices will affect both oral and gut microbiome; (2) the oral and gut microbiome will shift dynamically in a short period; and (3) the overall shift resulting from the changes in the gut microbiome members will be associated with known benefits to health and well-being. To achieve this goal, we performed targeted 16S rRNA gene amplicon-based sequencing on both fecal and oral samples of twenty-four participants at three different timepoints of the retreat. This study aims to demonstrate that Pranic Healing and Arhatic Yoga practices

combined with vegetarian diet promote the enrichment of healthy microbes. Findings from this study could be used as potential therapeutic interventions and preventions for improving and maintaining homeostasis and overall health.

Materials and methods

Recruitment of study participants and ethical statement

A subject must meet the following inclusion criteria to be eligible to participate in this study: (i) must provide written informed consent prior to initiation of any study procedures (ii) was able to understand and agreed to comply with planned study procedures (iii) agreed to the collection of specimens (saliva and fecal) before, during and after the study (iv) male or non-pregnant female greater than 18 years of age at time of enrolment and (v) must have a distinct Arhatic level (0–5 level) and performed meditation and MTH practices from once a week to more than 3 times a week prior to this retreat. A subject was excluded from the study if the following criteria were present: (i) concomitant disease that requires any medical treatment (ii) pregnancy, (iii) any antibiotic treatment the past two weeks (iv) pediatric population, (v) clinical apparent infectious disease. We calculated the sample size for this study as performed in human microbiome studies [40]. Adopted from the sample size calculation table from this study, we required 15 subjects to have 80% power at $p < 0.05$ significance level. Based on the above inclusion and exclusion criteria, 27 healthy registered practitioners in this retreat were recruited for this study and their informed consent were obtained. Age, gender, and basic vital signs such as height, weight, and body temperature were measured for each such practitioner (Supplementary Table S1). Ethical approval (IRB No: 22–08–521–1247) was obtained from Pranik Healing Research Institute before the start of the study. This study complies with the guidelines for biofield therapies called Biofield Therapies: Reporting Evidence Guidelines (BiFi REGs) which was supplemented with CONSORT 2010 [38, 41, 42].

Intensive spiritual retreat protocol

The spiritual retreat was held in a 84-acre meditation center in Bovina, New York for a duration of 9 days. All practitioners had undertaken formal training on Pranik Healing, Meditation on Twin Hearts, and higher meditation practices at least six months prior to the retreat [43]. Daily retreat practices lasted typically for 16–18 h with occasional short breaks. These practices included combinations of specifically designed physical and breathing exercises to improve the energetic states and stillness. Exercises were followed by Pranik Healing and self-awareness practices. Meditations included Meditation

on Twin Hearts [43, 44], Arhatic Yoga meditations of Kundalini meditation, Arhatic Dhyana and Meditation on the Higher Soul [43]. The schedule of the retreat (Supplementary Table S2) and daily meal plan (Supplementary Table S3) are provided in supplementary. The participants followed an identical vegetarian diet, with no recent histories of smoking, alcohol intake and illicit drugs. There was open communication between all the participants before, during and after the retreat. As all the practitioners followed group practices in one location under the supervision of the senior instructors, separate assessment of the adherence of the practitioners to the protocol was not performed.

Sample collection, DNA extraction, amplification, and amplicon sequencing

Oral and fecal samples of 24 practitioners were collected at the start (Day0: T1), middle (Day3: T2) and end (Day9:T3) of the retreat in a sterile container and sent for DNA extraction and sequencing to Metagenom Bio Life Science Inc, Waterloo, Canada. Being a single-arm, time-stamped study, no control groups were present, however, the timepoints served as comparators. Participant's information was only available to the logistic coordinator. Samples were therefore blinded for DNA sequencing. For analysis and interpretation participant metadata as presented in Supplementary Table S1 were used, where participant name remained blinded. Total genomic DNA from stool and saliva was extracted with the Sox DNA Isolation Kit (Metagenom Bio Inc., Cat. no. 18011_S0) according to the supplier's recommendation. The DNA was further subjected to amplification of V4 region of the 16S rRNA gene with primer set (515FB: 5'-GTG YCAGCMGCCGCGGTAA-3' and 806RB: 5'-GGACTA CNVGGGTWTCTAAT-3') [45]. The PCR was set in triplicates for each sample (25 µl each). Each reaction mixture contained 2.5 µl of 10× standard Taq buffer, 0.5 µl of 10 mM dNTP, 0.25 µl of BSA (20 mg/ml), 5.0 µl of 1 µM forward primer, 5.0 µl of 1 µM reverse primer, 5.0 µl DNA, 0.2 µl of Taq DNA polymerase (5u/µl) and 6.55 µl of PCR water. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 30 °C for 30 s and 72 °C for 50 s and then extended at 72 °C for 10 min. The triplicate PCR products were pooled and resolved with 2% TAE agarose gel. Equimolar pool of amplicons was prepared, quantified using Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific Inc.) and was sequenced with MiSeq Reagent Kit v2 (2×250 cycles).

16S rRNA gene sequence analysis and statistics

The raw sequences obtained after sequencing were subjected to quality checking using FastQC [46]. The

pre-processing and analysis were performed using Divisive Amplicon Denoising Algorithm 2 (DADA2) package v1.6.0 [47]. Primers and low-quality bases were removed from the end of the reads using filter and Trim function of DADA2. Non-chimeric and error-free amplicon sequence variants (ASVs) were generated and subjected to taxonomic assignments using SILVA Database (silva_nr-99_v138.1_train_set.fa.gz) [48, 49]. Decontam package was used to remove contaminants from the datasets using prevalence-based method [50]. The rarefaction curve was generated using rarecurve function of vegan package [51]. Both alpha (which deals with the richness, evenness, or both) and beta diversity (which compare the species diversity in two different ecosystems) metrics were generated by Phyloseq v3.4.2 R package [52]. Differences in alpha diversity metrics (which deals with the richness, evenness, or both) were tested using pairwise Wilcoxon test. Non-metric multidimensional scaling (NMDS) was performed with Bray–Curtis's dissimilarity matrix to understand the difference in the microbial community composition between the sampling sites (oral and gut), while principal coordinate analysis (PCoA) was performed to assess the change in the microbiome profile of the participants with time and different practices. Pairwise permutational ANOVA (PERMANOVA) with adjusted *p*-value was performed using the Bray-Curtis dissimilarity matrix to assess the difference in beta diversity using pairwise.adonis function. DESeq2 [53] analysis was performed to determine the differentially enriched or depleted ASVs during the retreat using Wald test with an adjusted *p*-value filter of $p < 0.01$. The predicted

metabolic function of the gut microbiome was performed using the phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) [54] followed by identification of statistically significant differentially abundant functional KEGG categories/metabolisms between participants with time using STAMP software [55] implemented with Welch's *t*-test and *p*-values were adjusted by Bonferroni correction. The raw sequences were submitted to the NCBI under Bioproject number PRJNA1071264. No changes were made to the trial outcomes after the trial commenced.

Results

Alpha and beta diversity of gut and oral microbiome of the practitioners

Targeted 16S rRNA gene-based amplicon sequencing was performed to assess the change or modulation in both gut and oral microbiome profiles of the participants during the retreat. In total, 24 fecal samples and 11 saliva samples were finally considered for the downstream analysis which yielded ~3.7 and ~2.2 million raw reads, respectively. After quality filtering, ~3.04 and ~1.66 million reads from fecal and saliva samples were obtained, respectively. These reads resulted in ~132 ± 38 and 84 ± 17 ASVs per fecal and saliva samples, respectively. The rarefaction curves of all analyzed samples attained saturation or plateau, indicating that the sequencing depth was sufficient to capture the maximum microbial diversity (Supplementary Fig. S1). The results further revealed that the alpha diversity measures (Shannon and Simpson

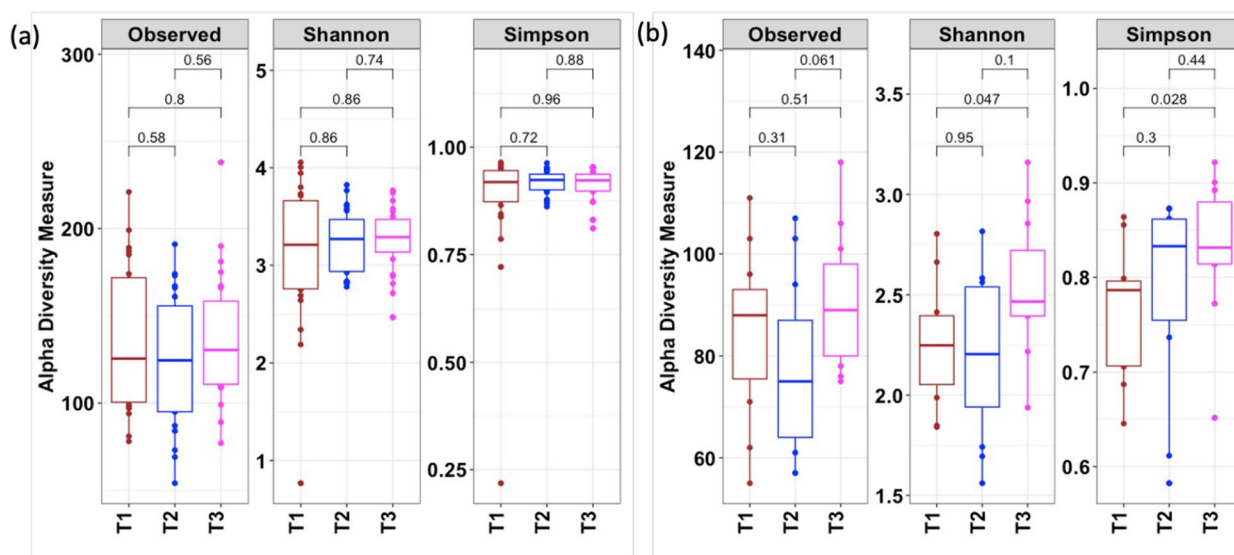


Fig. 1 Alpha diversity measures of the fecal and oral microbiome. Timepoint variations in the alpha diversity measures of (a) fecal and (b) saliva samples. Pairwise-Wilcoxon test was performed to assess the significant difference in the alpha diversity parameters over time

index) of the gut microbiome did not show significant ($p > 0.05$) differences over time (Fig. 1a). On the contrary, oral microbiome profile showed a significant ($p < 0.05$) difference in the species richness and evenness at the end of the retreat (Fig. 1b). However, gut microbiome was richer and more diverse or complex than oral microbiome (Supplementary Fig. S2). Moreover, non-metric multidimensional scaling (NMDS) uncovered the difference in the microbiome profiles of oral and gut microbiome (Fig. 2). PERMANOVA analysis ($p < 0.05$) further supported the difference in the oral and gut microbiome structure. It was interesting to note that the gut microbiome profile of the practitioners varied over time as depicted from the PCoA analysis which was further supported by pairwise PERMANOVA analysis ($p < 0.05$ for T1 and T2; $p = 0.05$ for T1 and T3; $p > 0.05$ for T2 and T3) (Fig. 3a). Conversely,

such changes in the oral microbiome profile were not observed over time (pairwise PERMANOVA analysis: $p > 0.05$ for T1 and T2; $p > 0.05$ for T1 and T3; $p > 0.05$ for T2 and T3) (Fig. 3b).

Changes in gut and oral microbial community structure of the practitioners

The gut and oral microbiome structure of the practitioners at T1 timepoint was predominated by *Firmicutes* (gut: 81.25 ± 16.67 and oral: 76.72 ± 7.37) followed by *Actinobacteriota* (gut: 4.17 ± 3.85 and oral: 14.7 ± 7.9), *Bacteroidota* (gut: 12.74 ± 16.27 and oral: 4.73 ± 4.52), and *Proteobacteria* (gut: 1.01 ± 2.26 and oral: 2.59 ± 2.76) members (Supplementary Fig. S3). At the end of the retreat at T3 timepoint, a significant (Pairwise Wilcoxon test, $p < 0.05$) decrease in abundance of *Bacteroidota* (5.84 ± 8.39) members was observed in the gut, while a

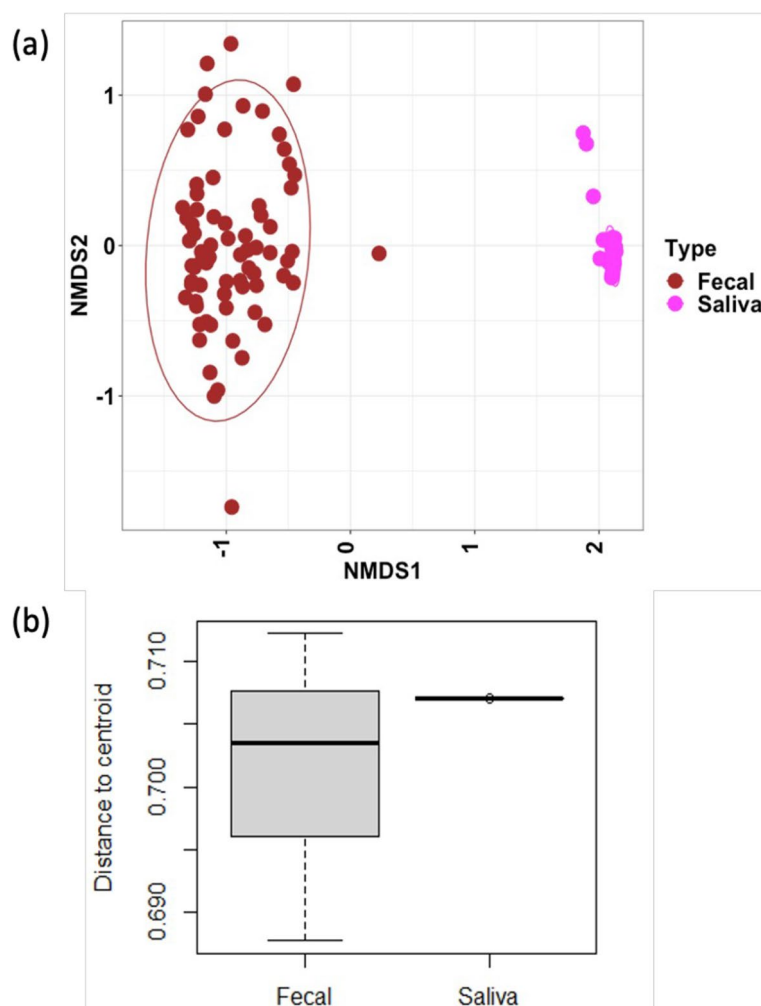


Fig. 2 Beta-diversity plot depicts difference in fecal and oral samples. **(a)** Non-metric dimensional scaling (NMDS) based on a Bray–Curtis dissimilarity matrix (Stress = 0.15) on microbiome profile of fecal and oral samples. **(b)** Distance to centroid is represented in box plot. Each dot represents the sample from each participant

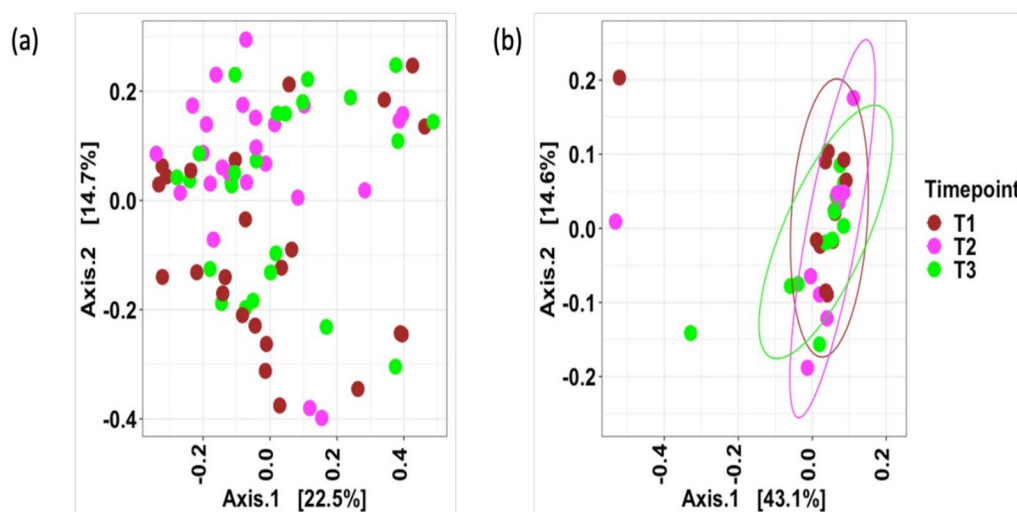


Fig. 3 Changes in microbiome profile of fecal and oral samples over time. Principal coordinate analysis (PCoA) ordination plot based on a Bray–Curtis dissimilarity matrix of microbiome profile of fecal (a) and oral (b) samples with time

significant (Pairwise Wilcoxon test, $p < 0.05$) increase in relative abundance of *Actinobacteriota* (9.55 ± 10.04) members in the gut of the practitioners was observed. Additionally, several microbial genera showed a prominent shift in their abundance patterns over time. Among them, relative abundance of *Ruminococcus torques* group, *Akkermansia*, *Pseudomonas*, *Romboutsia*, *Streptococcus*, *Subdoligranulum*, and *Ligilactobacillus* increased over time in gut of the practitioners and categorized them under persistent responders (Fig. 4). While *Blautia*, *Eubacterium halli* group, *Alistipes*, *Enterococcus*, *Clostridium sensu stricto 1*, *Bacteroides*, and *Anaerostipes* showed reduction in the relative abundance of the gut of practitioners from T1 to T3 (Fig. 4). In case of oral microbiome, *Abiotrophia* and *Porphyromonas* were the persistent responders, whereas the abundance of *Leptotrichia*, *Lachnoaerobaculum*, *Leuconostoc*, *Oribacterium*, and *Solobacterium* decreased from T1 to T3 (Fig. 5). Interestingly, there were microbes which showed transient response [increased in abundance was observed till T2, thereafter declined (T3)] in their abundance pattern during the retreat in gut (*Agathobacter*, *Bifidobacteria*, *Collinsella*, *Coprococcus*, *Dorea*, *Monoglobus*, *Fusicatenibacter*, *Ruminococcus*, *Anaerostipes*, and *Bacteroides*) and oral (*Granulicatella*, *Gemella*, *Pseudomonas*, *Rothia*, *Streptococcus*, and *Veillonella*) samples of the practitioners.

The specific alterations in gut microbiome associated with practice during the retreat over time were further resolved based on DESeq2 based analysis. Several differentially abundant ASVs were identified which showed significant ($p < 0.05$) changes in their abundance with time (T1 vs T3) (Fig. 6). Certain ASVs associated with

Erysipelotrichiaceae UCG-003 *Eubacterium halli* group, *Blautia*, *Faecalibacterium*, *Fusicatenibacter*, *Ruminococcus torques* group, *Akkermansia*, *Bifidobacterium*, *Pseudomonas*, *Romboutsia*, *Streptococcus*, *Subdoligranulum*, *Turicibacter*, *Ligilactobacillus*, *Alistipes*, *Enterococcus*, *Clostridium sensu stricto 1*, *Bacteroides*, and *Anaerostipes* showed prominent shift in their abundance at the end of the retreat (T1 vs T3), indicating the Arhatic Yoga meditation practices together with vegetarian diet had an impact on the modulation of certain groups of microbes to promote good health (Fig. 6). In order to assess the influence of age and gender in the gut microbiome changes, the participants were classified into three age categories (Category1: 30–45 years; Category2: 46–60 years; Category3: 61–75 years), two genders (male and female). Pairwise PERMANOVA comparisons between the categories and between the genders were not significantly different for their microbiome composition (Supplementary Table S4 and Table S5).

Predictive metabolic function of the gut microbes

The predicted functional pathways of the gut microbiome were determined through PICRUSt2 analysis. The results revealed that predicted functional KEGG categories/metabolisms showed an enrichment of various pathways such as cysteine and methionine metabolism, selenocompound metabolism, glycine, serine, and threonine metabolisms, sulfur metabolism, nicotinate and nicotinamide metabolism, and glycerophospholipid metabolism at the end of the retreat (T3 timepoint) (Fig. 7). However, folate biosynthesis, drug metabolism, pentose and glucuronate interconversions, and riboflavin metabolism were relatively reduced at the end of the retreat.

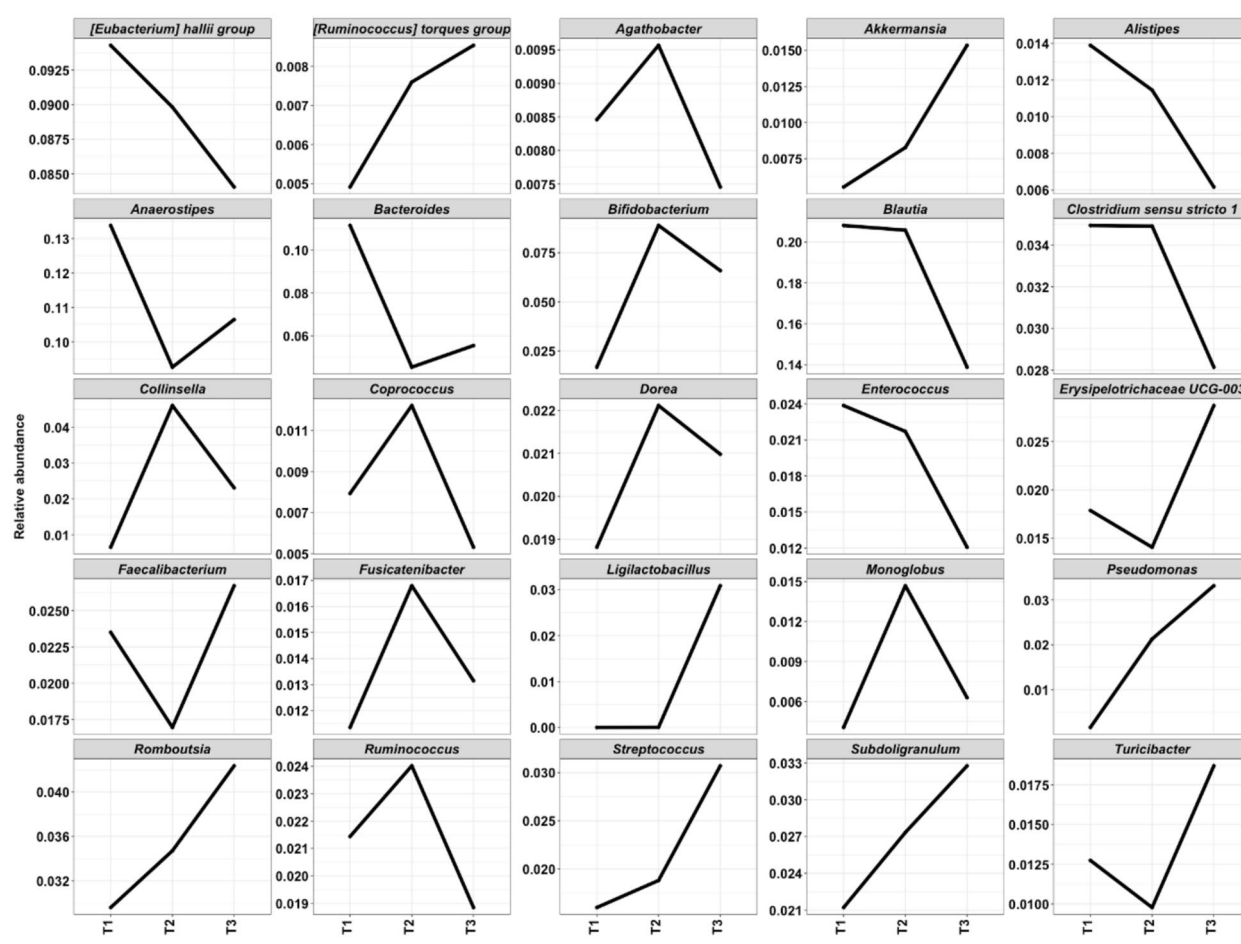


Fig. 4 Line graph representing the change in average abundance of major bacterial taxa (top 25) with time in fecal samples

Changes in the gut microbiome profile based on the Arhatic Yoga level, Meditation on Twin hearts (MTH), and meditation practices of the practitioners during retreat

Each practitioner who participated in the retreat already had a distinct Arhatic level (0–5 level) and performed meditation and MTH practices from once a week to more than 3 times a week. We further tried to understand the changes in gut microbiome profile of these participants during the retreat where they were exposed to the same environment, training, practices, and vegetarian food. The results revealed the slight convergence of the microbiome of the practitioners with higher Arhatic level (3 and above) at the end of the retreat (Supplementary Fig. S4a). Similarly, microbial profiles of frequent practitioners (3 or more times a week) of MTH and meditation converged more than non-frequent practitioners (Supplementary Fig. S4b and Fig. S4c). However, these results were not statistically significant ($p > 0.05$) as confirmed by PERMANOVA analysis. Furthermore, we

identified several differentially abundant ASVs ($p < 0.05$) which were associated with higher Arhatic level at T1 and T3 timepoints (Fig. 8). ASVs such as *Erysipelotrichaceae* UCG-003 *Eubacterium hallii* group, *Blautia*, *Faecalibacterium*, *Fusicatenibacter*, *Ruminococcus torques* group, *Akkermansia*, *Bifidobacterium*, *Pseudomonas*, *Romboutsia*, *Streptococcus*, *Subdoligranulum*, *Turicibacter*, *Ligilactobacillus*, *Alistipes*, *Enterococcus*, *Clostridium sensu stricto 1*, *Bacteroides*, and *Anaerostipes* predominated in the practitioners with higher Arhatic level at T3 timepoint.

Discussion

Yoga and meditation practices, physical activities, and exercises have been reported to improve gastrointestinal disorders and mental health [30, 56–60]. Arhatic Yoga is a global non-sectarian spiritual practice which cultivates holistic development of the physical, emotional, and mental aspects [39, 43]. The impact of meditation and vegetarian diet on the modulation of gut and oral

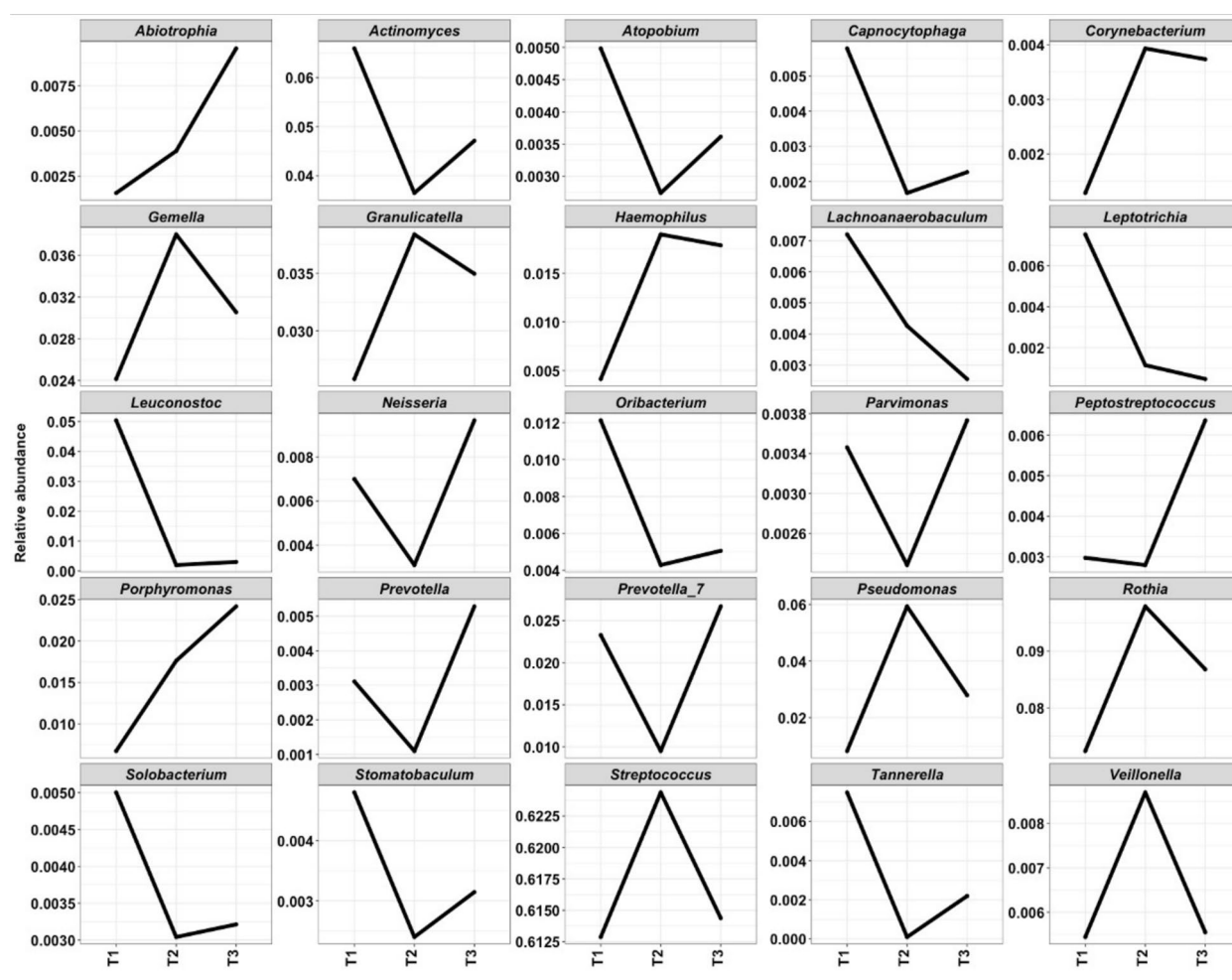


Fig. 5 Line graph representing the change in average abundance of major bacterial taxa (top 25) with time in oral samples

microbiome is not well understood. This study is the first of its kind in two respects. Firstly, to assess the impact of intensive meditation practices on the modulation of microbiomes in a controlled environment, where all the participants followed the same lifestyle and practices. Secondly, to demonstrate the associations of meditations in a short span of 9 days on the oral and gut microbiome leading to better understanding of its effects. This pilot study demonstrated that Arhatic Yoga meditation together with a vegetarian diet enriched the health-benefiting microbes both in the gut and oral environments. Hence, meditation could be incorporated in our day-to-day life to modulate our oral and gut microbiome for health benefits.

Our findings suggested that the oral microbiome had a more pronounced effect on alpha diversity parameters than gut microbiome profile at the end of the retreat. This might be due to the microbes that were acquired during the retreat practices from the combination of

lifestyle and diet. Although there were no overall changes in beta-diversity in the case of oral microbiome, several health beneficial microbes were enriched in the oral microbiome structure during the retreat. Most noteworthy are *Corynebacterium*, *Rothia*, and *Neisseria*, which are known for the nitrate-nitrite-nitric oxide pathway that plays a significant role in providing oral microbiome-mediated nitric oxide signaling molecules for cardiovascular or metabolic health [61, 62].

Our results showed that the gut microbiota composition of the participants changed in the short-term span, indicating that Arhatic Yoga meditation together with a vegetarian diet had substantial influence on gut microbiota composition. The effect of the changes in the composition of the microbiota on health and well-being can be supported by the predicted enrichment of certain microbial metabolic pathways such as the amino acid metabolism of selenocompound, cysteine, methionine, glycine, serine, and threonine. Similarly, the predicted

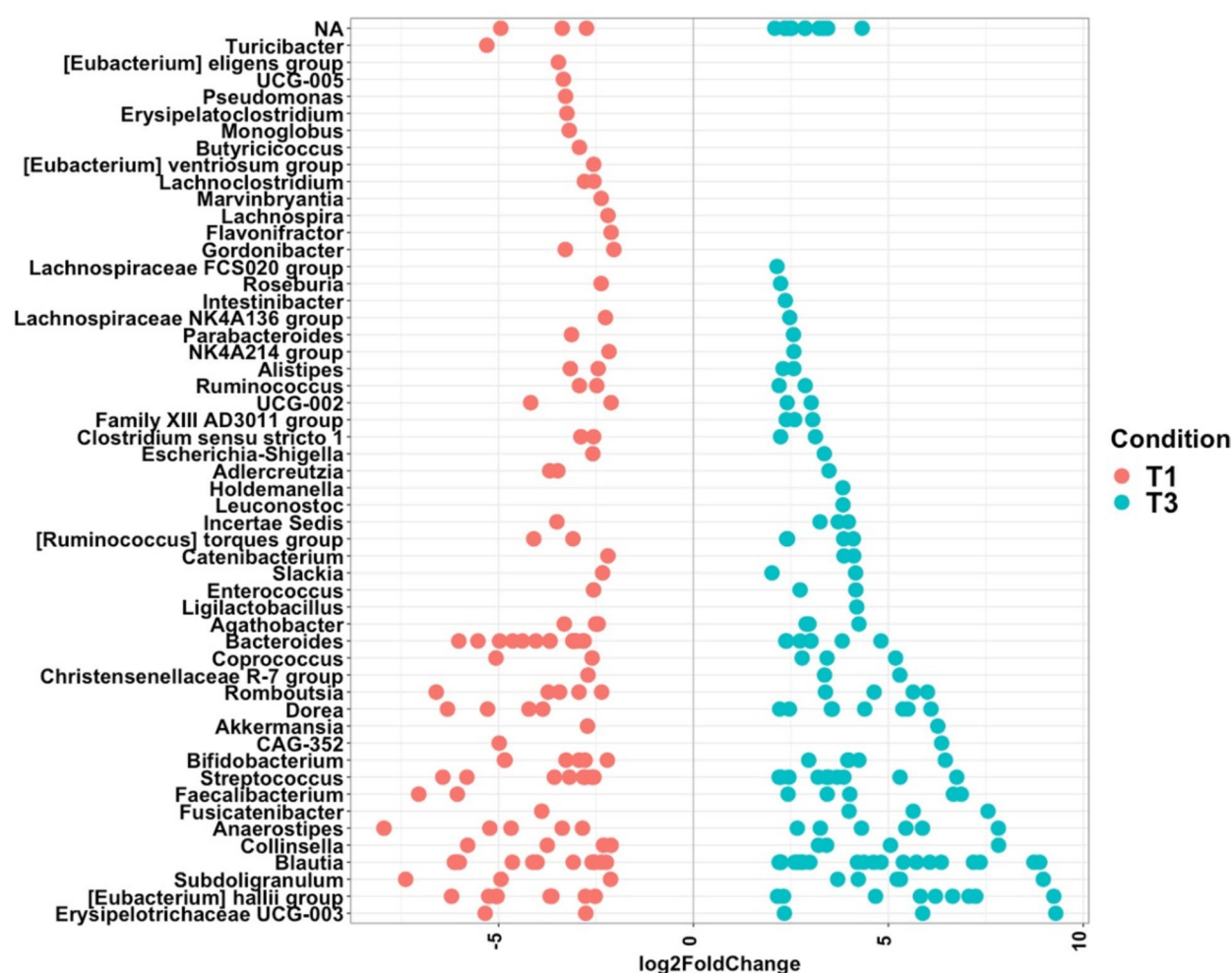


Fig. 6 DESeq2 based identification of major microbial ASVs ($p < 0.05$) which are differentially abundant between timepoint T1 and T3 in fecal samples

enrichment of nicotinate and nicotinamide metabolism, glycerophospholipid metabolism and chlorocyclohexane and chlorobenzene degradation indicated potential detoxification benefits. Nicotinate and nicotinamide are precursors of the coenzymes nicotinamide-adenine dinucleotide (NAD⁺) and nicotinamide-adenine dinucleotide phosphate (NADP⁺) which in their reduced form, NADH and NADPH respectively, are important co-factors in many redox reactions, including the chemical modification of DNA, RNA, and certain proteins [63, 64]. Methionine plays a role in the activation of certain antioxidant enzymes and biosynthesis of glutathione, which is key in counteracting oxidative stress [65]. Physical activities have previously shown the changes in amino acid metabolism, which might play a role in redox balance, oxidative stress, and immune function [66–68]. Glycerophospholipids are critical components of neuronal membranes and myelin, and principal regulators of

synaptic function and might play crucial role in gut-brain axis [69].

Furthermore, we identified several bacterial members that differed significantly with time. Increased abundance of *Erysipelotrichaceae* UCG-003, *Ruminococcus torques* group, *Subdoligranulum*, *Ruminococcus*, *Fecalibacterium*, *Roseburia*, *Akkermansia*, *Romboutsia*, and *Bifidobacterium* in the present study is in line with previous studies associated with gut microbiome profile of high-intensity competitive training of athletes or body builders, marathon runners, and moderate physical exercises or training along with vegan meditation and mindfulness cognitive therapy [8, 26, 70–78]. *Ruminococcus torques* group; *Subdoligranulum*; *Erysipelotrichaceae* UCG-003, *Ruminococcus*, *Fecalibacterium*, and *Roseburia* are the SCFAs (short-chain fatty acid) producing microbes and play significant roles in the immune modulation as well as in gut-brain axis [79–81]. SCFAs are known to

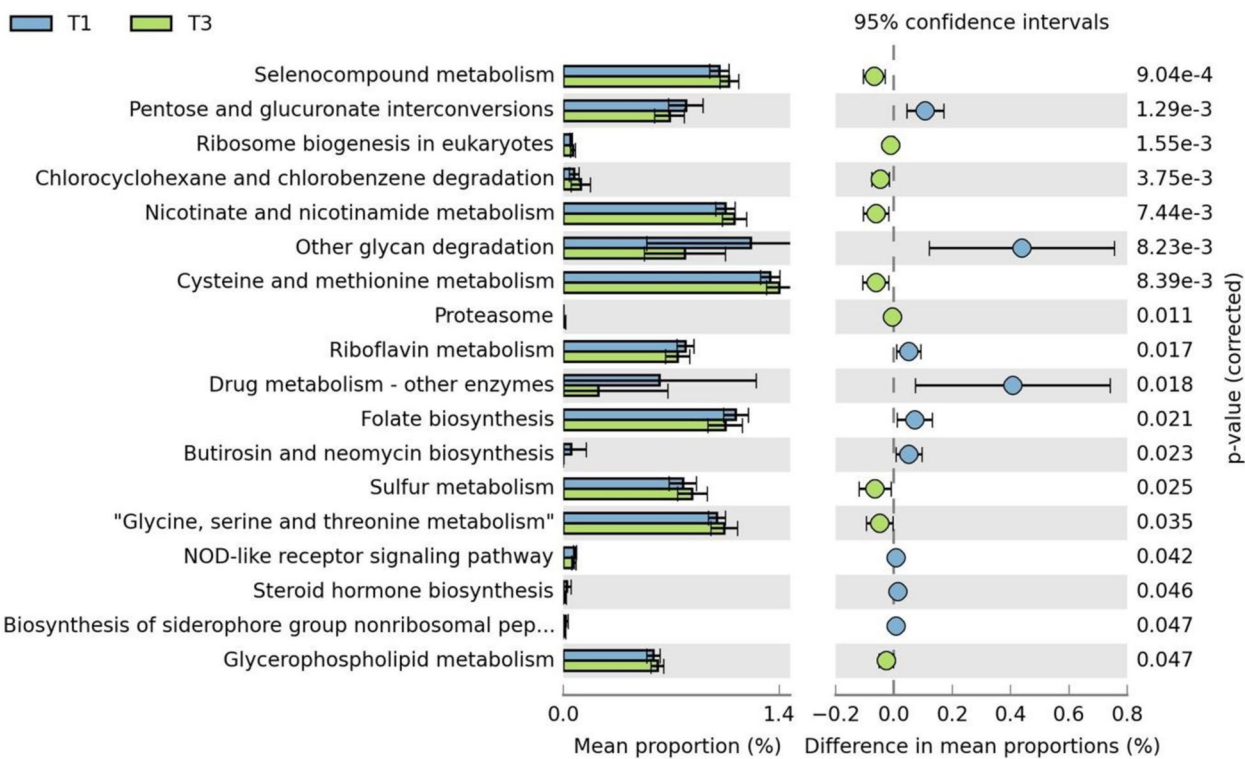


Fig. 7 Differentially abundant KEGG derived metabolic pathways of fecal samples between T1 and T3 timepoints

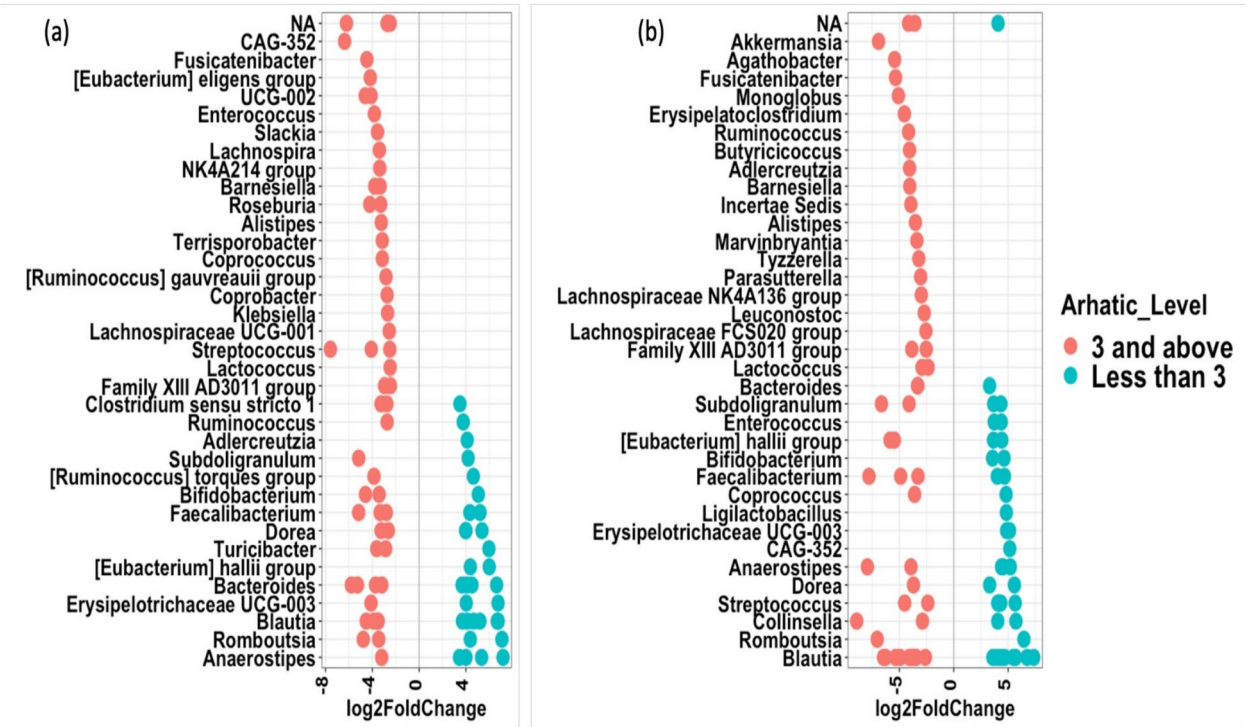


Fig. 8 DESeq2 based identification of major bacterial ASVs ($p < 0.05$) which are differentially abundant between practitioners with different Arhatic levels at timepoint T1 (a) and T3 (b) in fecal samples

play significant roles locally in improving gut health. For example, it maintains gut barrier integrity and protects from intestinal inflammation [80, 82]. SCFAs are also recognized as a key play in gut-brain axis and influence gut-brain function through endocrine, immune, vagal, and humoral pathways [82]. Hence, increased abundance of the above-mentioned SCFAs producing genera in the present study might play a significant role in modulating the gut-brain axis. *Akkermansia* species participate in the gut-barrier functions and are linked with a healthier metabolic status of the human body [83]. *Ligilactobacillus*, *Romboutsia* and *Turicibacter* have been reported in other non-meditation studies to have health-benefiting effects. *Ligilactobacillus* species are known to protect intestinal barrier [84] and recently, *Ligilactobacillus salivarius* was used as probiotic which improved the body growth and antioxidation capacity via microbiota-gut-brain axis in non-human model [85]. It has been reported that *Ligilactobacillus salivarius* also modulates the behaviour in non-human autistic model through microbiota-gut-brain axis [86]. *Romboutsia* species are involved in amino acid metabolism which might be positively associated with the gut-brain axis [87, 88]. For instance, there have been several reports where abundance of *Lachnospiraceae*, *Romboutsia*, and *Faecalibacterium* genera reduced in patients with anxiety, depression, and autism which influenced neural, immune, and endocrine pathways [87, 89–91]. Increased abundance of these genera in the present study further supported that Arhatic Yoga meditation with vegetarian diet improved the gut microbiome composition by enriching the beneficial microbes which could be involved in improving the gut-brain communications and mental health. Interestingly, the Meditation on Twin Hearts that was practiced daily during this study period produces significant changes on the brain qualitative electroencephalogram, particularly increase in P300 response times and gamma waves [44]. Hence, Meditation on Twin Hearts could have additionally contributed to the gut-brain axis in a positive manner.

As such, these meditation practices could be implemented into the regular lifestyles to improve gut microbiota composition. This in turn, promotes good health by reduction in inflammation, and gut-barrier permeability along with improvement of gut-brain function. Our data further suggests that frequent practitioners of Meditation of Twin Hearts and higher Arhatic meditation practices showed the convergence of microbiome profile with increased abundance of health-promoting microbes which results from a controlled practices during the retreat. Whereas practitioners with the lower arhatic levels and infrequent meditation practices also showed shift in their gut profile and tend towards the convergence not as evident in case of higher arhatic

level practitioners. Further investigations are required to delineate the effect of diet and meditation practices on such changes as multiple confounding variables are associated with such observations.

Overall, our study suggested that Arhatic Yoga practices together with vegetarian diet showed prominent effects on both the gut microbiome and the oral microbiome in a noticeably short span of time. However, the study has certain limitations such as a single-arm study with small size of datasets and lack of metabolite profiles of practitioners. Future studies may build from this work to deconvolute the effect of diet and meditation practices from the overall responses as well as long-term effect of these on the well-being of the participants through multi-omics approaches. Future studies will be designed with multiple controls to delineate the diet and meditation effect on the gut microbiome. The microbial metabolites linked with the regulation of immune response and neuromodulation will also help us to better understand the gut-brain axis. However, metatranscriptomics/metagenomics would further help in identifying the differences in expression profiles of the microbiome over time in response to these practices.

Conclusion

This study demonstrated that Arhatic Yoga meditation together with a vegetarian diet during a short intensive retreat resulted in increased alpha diversity of oral microbiome and beta diversity of the gut microbiome. Known health-promoting microbes were associated with these rapid changes. Such microbial consortia may be developed and used as probiotics to accelerate the benefits of both gut and oral microbiome functions.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12906-025-04783-4>.

Additional file 1.

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Authors' contributions

The study was designed by SS and GM. MC, VD, SS, and GM were responsible for coordination and implementation of the study protocol. MC, VD, and GM were responsible for sample collection and transport. MDJL, JC, and TCC were involved in study design, sampling strategy, DNA extraction, DNA sequencing and quality assurance. AG provided support on sequencing data analysis. AG with help of SS analyse the datasets. AG and SS involved in initial draft preparation. AG, SS, VR, GM, and MC, were involved in finalizing the final draft. All authors read and approved the final manuscript.

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Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

Ethical approval (IRB No: 22–08–521–1247) was obtained from Pranik Healing Research Institute before the start of the study. Informed consents were obtained from the participants.

Consent for publication

All authors have provided their consent.

Competing interests

The authors declare no competing interests.

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