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Human Microbiota in Contemplative Martial Arts: Gut Microbial and Oral–Salivary Profiles Across Tai Chi and Aikido Practitioners

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1 ABSTRACT

This study investigates the role of the microbiota in sport and health, with particular emphasis on Tai Chi and Aikido as meditative movement practices. The study addresses an important gap in exercise–microbiome research, where most available evidence has focused on elite or high-intensity athletic populations, while moderate and meditative forms of movement including movement combined with meditation ,remain underexplored. By integrating systematic reviews, meta-analysis, cross-sectional human studies, oral microbiota profiling, salivary biomarker assessment, and genome-resolved metagenomic analysis, this thesis examines how exercise intensity, meditation depth, salivary biomarkers, and sex-related biological variability contribute to faecal and oral microbial patterns associated with physical activity and health.

The systematic reviews and meta-analysis demonstrate that exercise is a relevant modulator of gut microbial ecology, with training intensity emerging as a major determinant of microbial organization. The evidence synthesis indicates that higher-intensity physical activity is associated with broader shifts in gut microbiota composition, including changes in the *Firmicutes/Bacteroidetes ratio (*currently reclassified as *Bacillota/Bacteroidata)* and microbial community diversity. At the same time, the narrative review on Ramadan intermittent fasting highlights the importance of nutritional timing, hydration, sleep, and recovery in shaping exercise-related physiological responses, while the review on sex-related microbiota differences shows that sex contributes to microbial variability in a context-dependent manner rather than as an isolated determinant. Together, these findings support the view that exercise-related microbial variation must be interpreted within a broader behavioural and physiological framework.

The original studies validate and extend these observations by showing that meditative movement may influence microbial composition in ways that are not explained by exercise intensity alone. Among Aikido practitioners, meditation depth was more strongly associated with gut microbiota variation than practice level, with compositional shifts involving genera such as *Bacteroides*, *Faecalibacterium*, and *Prevotella*. In the combined Tai Chi and Aikido cohort, meditation level and the interaction between sport type and meditation depth showed stronger associations with beta diversity than with alpha diversity, indicating that meditative engagement may primarily influence microbial community restructuring. Tai Chi and Aikido also displayed distinct microbial signatures, suggesting that different contemplative disciplines

may shape the gut in different ways. The oral microbiota study further extended these findings beyond the gut, showing structured beta-diversity patterns according to meditation and sport level, while lower salivary MMP9 concentrations in the higher sport-level group suggested a possible link between sustained practice and reduced inflammatory tone. In parallel, the metagenomic analysis confirmed that higher-intensity athlete groups exhibited greater microbial diversity and clearer ecological separation from sedentary controls.

The discussion highlights the dual relevance of these findings for sport and health sciences. On one hand, physical intensity appears to be a robust driver of microbial diversity and community structure in athlete populations. On the other, meditation-based movement practices such as Tai Chi and Aikido suggest that psychophysiological dimensions, including meditation depth, attentional regulation, and stress modulation, may also shape host–microbe interactions in meaningful ways. This thesis therefore broadens the conceptual scope of microbiome research beyond performance-centred sport and establishes a foundation for future studies integrating movement practices, host biomarkers, and microbial function to promote gastrointestinal, metabolic, oral, and psychological health.

2 INTRODUCTION

2.1 BACKGROUND

2.1.1 Microbiota and Human Health

The human body hosts a vast and dynamic community of microorganisms, including bacteria, fungi, viruses, and archaea, which colonize multiple anatomical sites such as the gut, oral cavity, skin, and lungs. Collectively, these microbial communities form the human microbiota and contribute functions that are essential for health and physiological stability. The term "microbiota" can be traced back to the early 1900s. A large variety of microorganisms, such as bacteria, yeasts, and viruses, exist in different areas of the human body including gut, skin, lung, and oral cavity (Ursell et al., 2014). The human gut microbiota includes phages, viruses, and archaea, primarily *M. smithii*, in addition to bacteria and fungi (Lozupone et al., 2012). The microbiota composition varies across different sites of human body (Figure 1) (Hou et al., 2022).

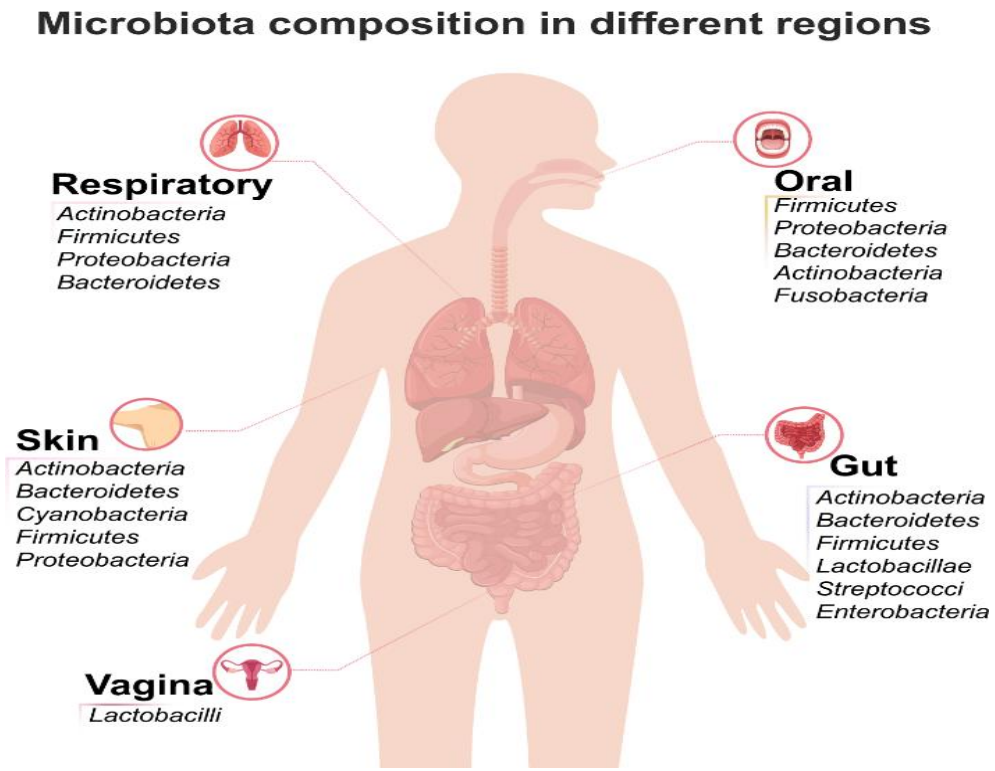


Figure 1: Microbiota composition in different regions (Hou et al., 2022)

Furthermore, the human microbiota, referred to as "the hidden organ," provide genetic information exceeding that of the complete human genome (Grice & Segre, 2012). Although "microbiota" and "microbiome" are frequently used interchangeably, there are notable distinctions between the two concepts. Microbiota refers to the living microorganisms present

in a specific environment, like oral and gut microbiota. Microbiome denotes the aggregation of genomes from all microorganisms present in the environment, encompassing not just the microorganism community but also the microbial structural components, metabolites, and environmental factors (Berg et al., 2020). Among all microbial habitats in the body, the gastrointestinal tract is regarded as the most important in relation to general health and disease (Shreiner et al., 2015).

The gut bacteria perform a number of tasks, including food fermentation, immune response stimulation, pathogen defence, and vitamin manufacturing (Hillman et al., 2017). *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria*, and *Verrucomicrobia* are the six major phyla that make up the gut microbiota but *Firmicutes* and *Bacteroidetes* are considered the two most major phyla among them (Laterza et al., 2016). *Candida, Saccharomyces, Malassezia*, and *Cladosporium* are the most researched and studied fungi (gut mycobiota). Microbiota is also found in other areas, such as the skin, oral cavity, lung, and vagina, but it is less well-established than in the stomach. The second-largest microbial population in humans is thought to be the oral microbiota (Deo & Deshmukh, 2019). The oral cavity can be further classified into several microbiota habitats, such as saliva, tongue, tooth surfaces, gums, buccal mucosa, palate, and subgingival/supragingival plaque. These habitats may show significant and quick changes in composition and activity due to things like pH shifts, gene mutations, and bacterial interactions. Although there are some variations, the microbiota makeup in each of the seven locations is generally comparable. *Firmicutes, Proteobacteria, Bacteroidetes, Actinobacteria*, and *Fusobacteria* are often the main bacteria found in oral microbiota.

Numerous investigations have shown that microbiota is also present in lung tissues, despite the long-held belief that healthy human lungs are sterile (Dickson & Huffnagle, 2015). *Actinobacteria, Bacteroidetes, Firmicutes,* and *Proteobacteria* comprised the core lung microbiota. Three main factors influence the composition of lung microbiota: 1) microbial immigration; 2) microorganism elimination; and 3) microorganism reproduction rates (Dickson et al., 2014). Each geographical area has a different distribution and variety of glands and hair follicles in human skin. Different skin regions have different microbiota compositions due to their physical and chemical variations. In general, *Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes*, and *Proteobacteria* make up the skin microbiota (Tong et al., 2018).

This wider perspective is useful because it highlights a central principle of microbiome science: microbial communities are highly context-dependent. Nevertheless, the gut remains especially

significant because of its microbial density, metabolic capacity, and close relationship with nutrition, immunity, and systemic physiology.

2.1.2 Healthy Gut Microbiota, Eubiosis and Dysbiosis

A healthy gut microbiota is usually described in terms of resilience, diversity, gene richness, and functional stability. Rather than being defined by the presence of one ideal microbial composition, gut health is better understood as a dynamic and relatively balanced state in which microbial communities coexist symbiotically with the host and support key physiological processes. Human health and illness are directly related to the balance of intestinal microbes. The human gastrointestinal (GI) tract is home to about 100 trillion bacteria, making it a more abundant microbial ecosystem than other parts of the body (Gomaa, 2020). Numerous investigations have demonstrated the crucial connection between gut bacteria and fundamental human biological functions. For instance, recent research has demonstrated the strong relationship between human microbiota and immunity, metabolism, and nutrient extraction (Bouskra et al., 2008). Microbiota can influence biological processes in several ways. The microbiota is essential for the extraction of nutrients and energy from food because of its diverse metabolic genes that produce distinct, independent enzymes and biochemical pathways (Turnbaugh et al., 2006). The gut microbiota plays a major role in the creation of bioactive compounds like vitamins, lipids, and amino acids. In terms of the immune system, the human microbiota plays an important role in the development of the intestinal mucosa and immune system in addition to protecting the host from foreign pathogens by generating antimicrobial compounds (Rafii et al., 2008). Microbiota are intricate systems made up of trillions of microorganisms. With sophisticated sequencing methods and analytics, most of the microbiota-related research is now cantered on the link between microbiota compositional changes and various disease states. When exposed to external changes, the balance of the microbiota community can be disrupted, resulting in dysregulation of body systems and diseases, as illustrated in Figure 2 (Hou et al., 2022).

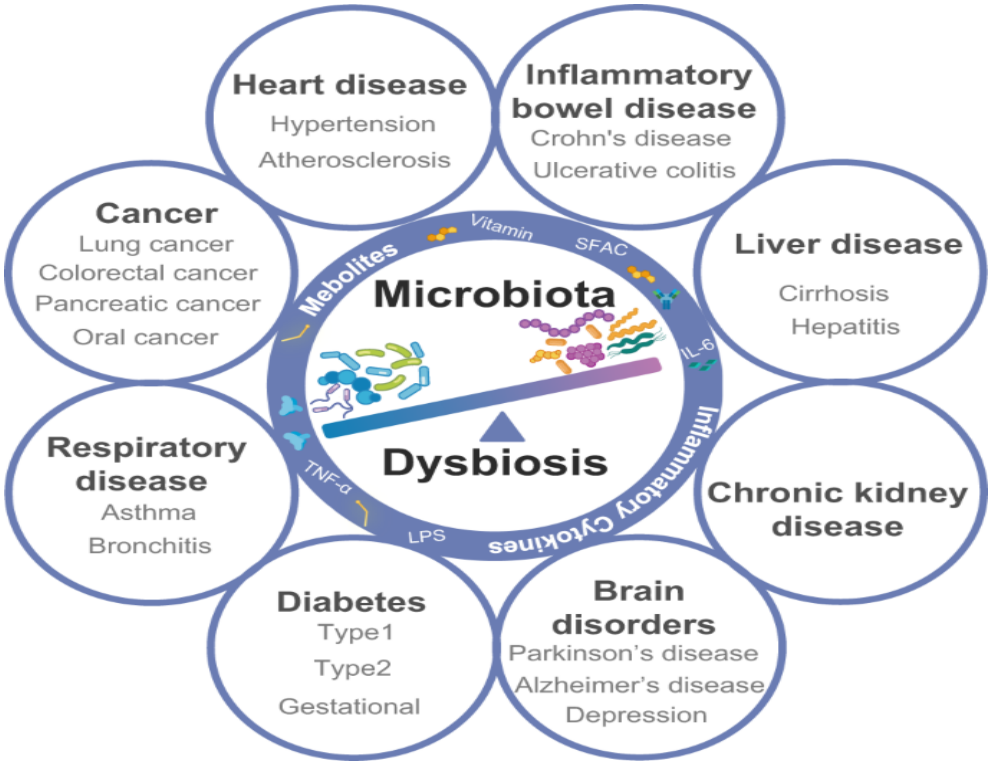


Figure 2: Human Microbiota dysbiosis contribution to diseases (Hou et al., 2022)

The gut microbiota shows resilience, stability, and symbiotic relationship with the host under normal circumstances. The notion of a "healthy" gut microbiota and its connection to host physiological processes have been extensively studied. Bacteria, yeasts, and viruses make up the gut microbiome. High taxonomic diversity, high microbial gene richness, and a stable core microbiota are frequently seen in a healthy microbiome community (Fan & Pedersen, 2021). It should be highlighted, nonetheless, that the relative distribution of microbes varies from person to person and even within the same person. Age and environmental factors (such as medication use) can affect a person's gut flora. Additionally, distinct GI tract anatomical regions have varied gut flora. Additionally, distinct GI tract anatomical regions have varied gut flora. For instance, the colon does not include *Proteobacteria* like *Enterobacteriaceae,* which are prevalent in the small intestine. Rather, the colon is frequently home to *bacteriodetes* such *Bacteroidaceae, Prevotellaceae,* and *Rikenellaceae* (Flint et al., 2012). These differences are mostly caused by the various environments. The colon has slower flow rates, a gentler pH, and larger microbial communities particularly anaerobic ones than the small intestine, which has short transit times and high bile concentrations (Milani, 2017). In addition to geographical dispersion, age also affects gut microbiota. In general, the diversity of the microbiota rises between childhood and adulthood and falls as people age beyond 70 (Rinninella et al., 2019). The environment, diet, and host genotype all affect the makeup of our microbiota. The microbiota's metabolic products and signalling chemicals affect a number of intestinal visceral

sensing, motility, digestion, secretion of permeability, energy harvesting, mucosal immunity, and barrier impact (Carding et al., 2015). Additionally, the components of the microbiota and/or microbiome may enter the bloodstream and travel to other organs that have an impact on their functionality, such as the pancreas (glucose metabolism), liver (lipid and drug metabolism), and brain (cognitive processes) (Korecka & Arulampalam, 2012). We become aware of the significance of the microbial ecosystem in preserving a healthy state when we understand that bacterial structural elements and/or metabolites are sufficient to promote the growth and maturation of organs and physiological processes in the host. Eubiosis, the equilibrium of the gut microbial environment, is a key idea. Hippocrates stated that "poor digestion is" and "death is in the bowels" as early as 400 B.C. Most diseases start in the digestive tract when the "good" bacteria are unable to control the "bad" ones, according to Ali Metchnikoff, who lived from 1845 to 1916. He named this state dysbiosis, which denotes an ecosystem in which bacteria no longer coexist peacefully.

Potentially beneficial species, primarily from the two bacterial phylum *Firmicutes* and *Bacteroides*, predominate in a gut microbiota in a eubiotic state, while potentially pathogenic species, such as those from the phylum *Proteobacteria* (*Enterobacteriaceae*), are present but make up a very small percentage (Zhang et al., 2015). Diseases and dysregulation of body processes can result from changes in the microbiome community's balance brought on by external stimuli. summarized in figure 2 (Hou et al., 2022). This variability is scientifically important because it means that microbial health cannot be judged solely by taxonomic presence or absence. Functional outputs, host context, and environmental influences must also be considered.

2.2 Physical Activity and Gut Microbiota

Among the lifestyle factors that shape the gut microbiota, physical activity has emerged as one of the most promising and biologically meaningful. Exercise is already well established as a protective factor in the prevention and management of chronic disease, but increasing evidence suggests that some of its benefits may be mediated, at least in part, through the gut microbiome. Engaging in physical activity, including both exercise and recreational activities, plays a crucial role in maintaining overall health and preventing or managing chronic diseases, particularly by impacting the gut microbiome (Cerdá et al., 2016). Research suggests that certain beneficial microbes, like those that produce short-chain fatty acids (SCFAs), are linked to regular exercise (Mailing et al., 2019). These microbes may help protect the intestinal lining, enhance mucus production, boost immune and metabolic functions, and even affect the gut-brain connection

(Gallè et al., 2019). Through these pathways, exercise may contribute not only to gastrointestinal health but also to reduced neuroinflammation, improved metabolic regulation, and enhanced psychological well-being. However, the relationship between exercise and the gut microbiota is not straightforward. Exercise effects appear to depend on intensity, duration, training history, and dietary context (Keohane et al., 2019). Moderate physical activity may support intestinal motility, reduce transit time, and encourage favorable microbial adaptation, whereas prolonged and intense endurance activity can place substantial physiological stress on the gastrointestinal system (Aya et al., 2021). Low-intensity exercise can decrease stool transit time, limiting the interaction between the microbiota and the mucus layer. In contrast, endurance activities can cause changes in the digestive system, such as reducing blood flow to the digestive organs by up to eighty percent (Warburton & Bredin, 2017). Long-duration exercise can have both positive and negative impacts, such as increased bacterial movement from the colon, enhanced gut permeability, and weakened gut barriers (Monda et al., 2017). These observations suggest that exercise should not be treated as a single undifferentiated exposure in microbiome research. Rather, the specific characteristics of training matter. The diversity of gut bacteria and the levels of SCFAs are influenced by both physical activity and diet. Studies show that daily aerobic exercise, lasting over an hour and performed at 60% of maximum heart rate, can significantly increase gut microbiota diversity and SCFA production (Dziewiecka et al., 2022). Diet also plays an important role in shaping gut health, especially high-fibre foods, proteins, unsaturated fats, and supplements, which all positively affect the gut microbiome. Athletes need to fuel their gut bacteria with the right diet and exercise to enhance performance. For example, endurance exercise impacts gut microbiota differently in athletes compared to non-athletes, with athletes experiencing a more noticeable effect (Hughes & Holscher, 2021). Exercise-related stress can alter gut microbiota structure, and studies exploring the relationship between diet, exercise, and gut health among athletes like bodybuilders and elite runners reveal differences in microbiota diversity based on sport type, diet, and individual athlete characteristics. Animal model studies also show that moderate-intensity exercise impacts both gut bacteria and metabolism. Furthermore, an observational study of elite athletes who followed high-protein, high-carb diets suggests that diet plays a role in shaping gut flora and impacting performance (Bonomini-Gnutzmann et al., 2022).

As a result, observed associations between exercise and microbial composition may partly reflect dietary confounding rather than exercise alone (Clarke et al., 2014). This is one reason why literature remains somewhat inconsistent. Some studies report increased diversity and

enrichment of specific bacterial families in active individuals, whereas others find modest or non-significant changes after training interventions (Davies et al., 2025). The complexity of these findings underscores the need for more nuanced approaches that account for exercise modality, intensity, duration, stress load, and accompanying lifestyle factors.

2.2.1 Microbiota in Athletes and Active Populations

Athletes' gut microbiomes are notably different from those of non-athletes, with greater microbial diversity and more SCFA production, which contributes to improved homeostasis, gut health, mental clarity, and reduced neuroinflammation. While scientific evidence continues to evolve, studies generally suggest that physical activity is associated with greater biodiversity in the gut, particularly in the *Firmicutes* phylum, which is more prevalent in active individuals compared to sedentary ones (O'Donovan et al., 2020). Controlled dietary studies have also highlighted how specific bacterial families, such as *Lachnospiraceae* and *Ruminococcaceae*, are influenced by exercise. However, more research is needed to understand the precise ways in which specific foods and supplements can influence the gut microbiota in response to exercise (Mitchell et al., 2019). Scientific research varies on several aspects, such as the types of changes in biodiversity and the relative impact of diet, physical activity, and other factors (Valeriani et al., 2020). A systematic review from 2021 found that most studies reported higher variability and more frequent presence of the Firmicutes phylum in physically active individuals compared to sedentary ones. Additionally, studies with controlled diets observed links between physical activity and specific bacterial families, such as *Lachnospiraceae*, *Clostridiaceae*, *Paraprevotellaceae, Ruminococcaceae*, and *Veillonellaceae* (Dorelli et al., 2021). An experimental study on young Caucasian adults also found a positive relationship between the *Firmicutes* phylum and body mass index (BMI). These patterns have been linked to improved homeostasis, gastrointestinal health, reduced neuroinflammation, and potentially better physiological adaptation to training. Nonetheless, the athlete literature is far from uniform, and broad conclusions should be made carefully. In 2021, Moitinho-Silva et al. investigated the effects of different physical activities, such as strength exercises and endurance training, on human physiology and gut microbiota. They found that these types of exercise have distinct, yet moderate, impacts on overall health, with specific changes in the gut microbiome. The study compared subjects who participated in endurance and strength training to those who did not engage in regular physical activity. The physically active group showed a significantly higher abundance of the genera *Coprococcus*, *Parasutterella*, and the family

Ruminococcaceae compared to the control group (Moitinho-Silva et al., 2021). Similarly, Jang et al. conducted a 2019 cross-sectional study with bodybuilders and distance runners, providing bodybuilders with a high-protein, high-fat, low-carbohydrate, and low-fibre diet, and distance runners with a low-carbohydrate, low-fibre diet. The results showed that bodybuilders on a high-protein, low-carbohydrate diet had lower gut microbiota diversity and a reduced abundance of short-chain fatty acid-producing bacteria, highlighting the combined role of diet and exercise in shaping gut microbial diversity (Jang et al., 2019). In 2017, Karl et al. conducted a randomized controlled trial to assess the effects of military training on gut microbiota, using three different groups that were given protein-based and carbohydrate-based diets and underwent four days of Arctic military training. They found that physiological stress impacted intestinal permeability, and dietary changes influenced the composition of the gut microbiota. Additionally, the Shannon and Chao1 diversity indices showed an upward trend (Karl et al., 2017). In a 2020 cohort study, Han et al. examined the gut microbiota of elite and non-elite athletes at juvenile and adult levels, analysing how exercise type, nutrition, and physical attributes influenced microbial communities (Han et al., 2020). In 2017, Yang et al. conducted a cross-sectional study on premenopausal women to explore the relationship between VO2-max and gut microbiota composition. They found that cardiorespiratory fitness and gut microbiota were linked, regardless of age or dietary fat and carbohydrate intake. The relationship between VO2-max and gut microbiota was largely influenced by body fat and physical activity levels (Yang et al., 2017). Oliveira et al. performed a cohort study in 2021 using the perceived exertion method to monitor changes in gut microbial diversity in female football players before and after a football tournament. The results indicated that the physical and physiological demands of training and matches in the official international tournament did not significantly alter the gut microbiota composition of elite female footballers, although the Shannon, Chao1, and Simpson diversity indices increased slightly. The abundance of *Firmicutes* and *Bacteroidetes* did not show much change before and after the tournament (Oliveira et al., 2022). Cronin et al. conducted a study in 2018 on athletes performing both aerobic and resistance training for 8 weeks. They found that improvements in body composition due to exercise were not significantly associated with changes in the gut microbiota composition (Cronin et al., 2018). In 2021, Barton et al. carried out a 6-month cohort study on male marathon and triathlon runners, who engaged in regular aerobic exercise combined with twice-weekly resistance training. They observed changes in gut microbiota and physiologically relevant metabolites because of consistent fitness training (Barton et al., 2021).

Collectively, these findings suggest that exercise can influence the gut microbiota, but the magnitude and direction of the effect vary substantially across populations and study designs.

2.2.2 Exercise Type and Intensity

The type, intensity, and duration of exercise have significant effects on the composition of the gut microbiota. Aerobic exercises, such as running and cycling, generally promote an increase in microbial diversity, whereas anaerobic exercises, such as weight training, may have different impacts. Additionally, high-intensity interval training (HIIT) and endurance training likely alter the microbiome in distinct ways, potentially optimizing energy metabolism and inflammation regulation, both of which are crucial for athletic performance (Bonomini-Gnutzmann et al., 2022). High-intensity interval training, endurance exercise, and moderate recreational activity are likely to shape the microbiome through distinct physiological routes, including differences in substrate use, inflammatory response, hormonal signaling, and gastrointestinal stress. In endurance sports, both a single session of exercise and prolonged training periods can impact the microbiota and overall health. Acute exercise sessions can be categorized into moderate and intense exercise, each having distinct effects on the body and gut microbiota, it is shown in Figure 3 (Clauss et al., 2021)

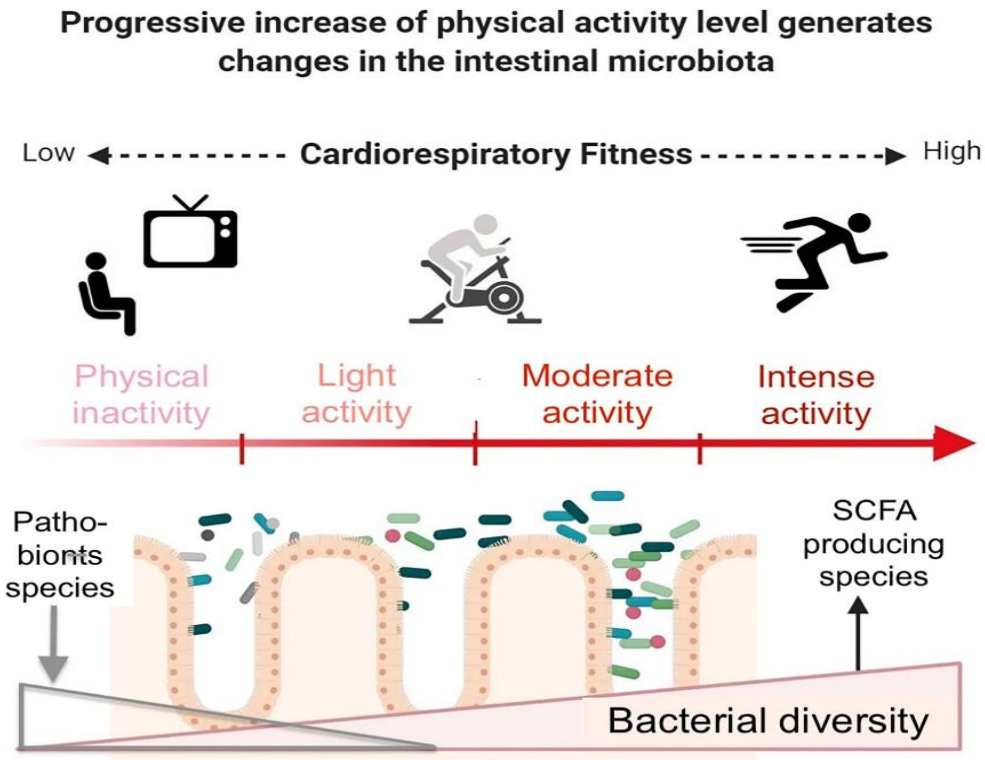


Figure 3: Beneficial effects of varied intensity exercises on gut microbiota (clauss et al., 2021)

A cross-sectional study conducted by kulecka on cross-country skiers and marathon runners who had undergone intense endurance training. They discovered that intense training was associated with changes in microbiota composition and increased bacterial diversity, with a particular emphasis on taxa involved in fibre fermentation (Kulecka et al., 2020). Tabone et al. conducted a clinical trial in 2021 with cross-country runners, involving different treadmill exercises at varying intensities. Their results showed significant changes in the microbiota, as well as in the serum and faecal metabolome, following a single exercise bout. These changes could have health implications for non-professional athletes (Tabone et al., 2021). In the same year, Craven et al. conducted a 7-week cohort study on middle-distance runners, examining how different training volumes influenced gut microbiota. The study found that changes in training volume did not significantly affect the alpha-diversity or overall composition of the gut microbiome (Craven, Cox, & Bellinger, 2022).

This point is particularly relevant when considering forms of movement that do not fit neatly into the categories of vigorous endurance training or strength sport. Most existing research has been dominated by athletes involved in performance-oriented activity, leaving relatively less attention to moderate, sustainable, and non-competitive forms of exercise. Yet these forms of movement may be highly relevant from both a health promotion and mechanistic standpoint. If moderate exercise influences the microbiota through pathways different from those involved in intense training, then excluding these populations narrows the field unnecessarily. It is within this context that the study of contemplative movement and martial arts becomes especially valuable.

2.2.3 The Gut Muscle axis and Brain-Gut-Muscle Communication

The intestinal microbiota plays a crucial role in the development and function of the central nervous system (CNS), as well as in regulating appetite and energy balance. It also influences skeletal muscle metabolism through metabolites like short-chain fatty acids (SCFAs) and bile acids (BAs), thus helping to maintain overall energy balance in animals. This framework is important because it shifts attention from microbial composition alone toward integrated physiological signaling networks that link metabolism, immunity, appetite regulation, and exercise adaptation.SCFAs promote AMPK phosphorylation in myotubes and skeletal muscles by increasing AMP concentration and the AMP/ATP ratio (Maruta et al., 2016). Phosphorylated AMPK activates downstream targets, including p38MAPK and PGC-1α, which enhances mitochondrial biosynthesis, function, and fatty acid oxidation (Jäger et al.,

2007). AMPK also boosts GLUT4 expression by phosphorylating HDAC5, leading to the release of HDAC5 from the nucleus in human primary myotubes. This process increases glucose uptake into muscle cells through GLUT4, enhancing muscle glycogen storage (Fernandez-Marcos & Auwerx, 2011). Beyond these pathways, SCFAs can bind to the GPR41 and GPR43 receptors, stimulating colonic L cells and pancreatic tissue to release GLP-1 and insulin, respectively. GLP-1 signalling activates PI3K and boosts GLUT4 protein levels in the cytoplasm, increasing glucose absorption. GLP-1 also enhances glucose uptake independently of PKB phosphorylation and promotes glucose storage without GSK3 phosphorylation (McGee et al., 2008). As a result, GLP-1 aids in muscle microvessel aggregation, glucose uptake, metabolism, and glycogen synthesis. Insulin further increases glycolysis by enhancing glucose transport and the activity of hexokinase and fructokinase 6-phosphate. Increased AMPK activity also reduces the expression of lipogenesis-related genes, such as ACC, SREBP-1c, and FAS, while promoting genes involved in lipid breakdown, such as CPT1, U, LPL, and HSL, leading to decreased fat accumulation in muscles (Richter & Hargreaves, 2013). Additionally, BAs act as signalling molecules that regulate TGR5 in muscles, promote thyroid hormone activation, and enhance energy expenditure in skeletal muscle cells (Green et al., 2012). BAs also prevent muscle fat deposition by activating the nuclear FXR. Overall, intestinal microorganisms produce metabolites that influence muscle lipid metabolism and glucose regulation, offering new perspectives for treating type 2 diabetes and obesity (Cipriani et al., 2010). Through these routes, intestinal microorganisms may influence muscular function in ways that are relevant to both performance and metabolic health.

Skeletal muscle is recognized as an endocrine organ due to the production and secretion of proteins called myokines by myotubes. Exercise appears to create a link between muscles, the intestines, and the central nervous system (CNS) shown in Figure 4 (Yin et al., 2022) . Skeletal muscle has the potential to produce hundreds of proteins, with IL-6 and IL-15 being particularly noteworthy (Ellingsgaard et al., 2011). Research showed that exercise increased the systemic IL-6 levels in mice, with skeletal muscle-derived IL-6 being the key mediator of this increase. Elevated IL-6 levels stimulate intestinal L cells to release GLP-1, which then acts on the CNS through the bloodstream, influencing appetite (Tekin et al., 2017). IL-6 can also directly impact the CNS to regulate appetite. Irisin, a novel myokine, is significantly upregulated during exercise. Studies indicate that central injection of irisin in rats increases food intake and ghrelin levels. It is suggested that irisin derived from muscle may stimulate gastrointestinal tissues to secrete ghrelin, thus influencing appetite regulation in the CNS. A recent study identified that exercise promotes the production of N-lactoyl-phenylalanine (Lac-Phe), a blood-borne

metabolite that inhibits feeding and prevents obesity . Several studies have also reported elevated plasma levels of PYY and PP, which can suppress hunger shortly after exercise (Martins et al., 2007). As a result, the phenomenon of "exercise-induced anorexia" may be linked to the increased levels of PYY, GLP-1, and PP observed during physical activity (Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009). While many studies suggest that myokines secreted post-exercise stimulate gastrointestinal hormone secretion and influence appetite through the CNS, the exact mechanism by which muscle cytokines interact with intestinal endocrine cells remains unclear, and further research is needed to elucidate the underlying mechanisms of this cross-talk (Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). This uncertainty creates an important opportunity for further research.

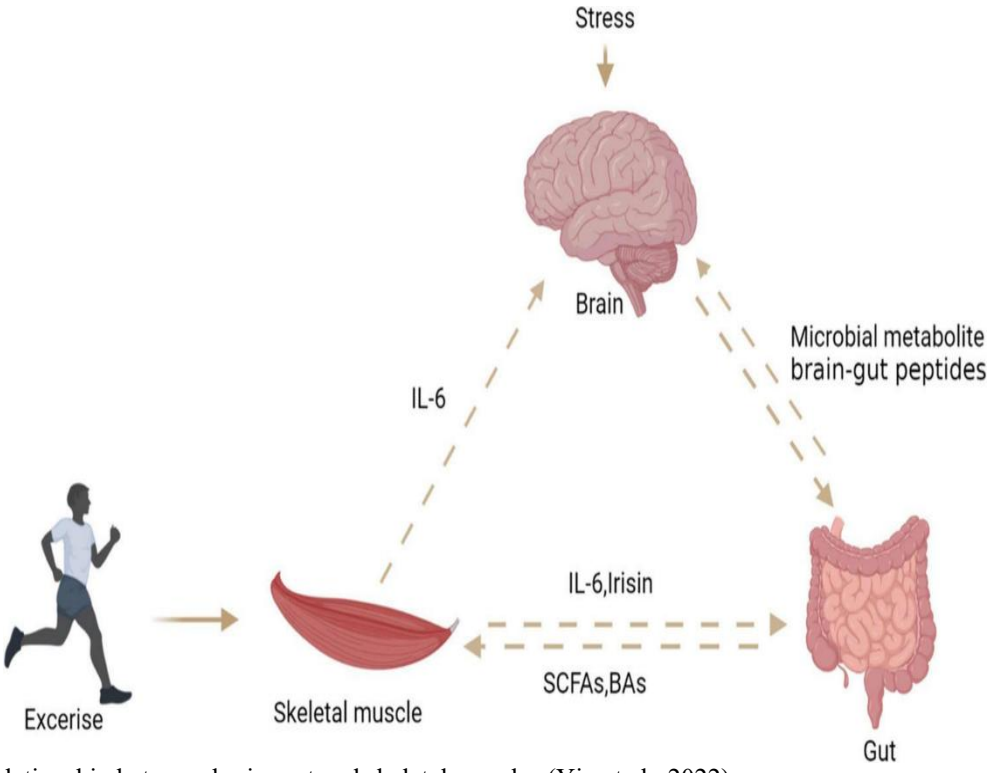


Figure 4: Relationship between brain, gut and skeletal muscles (Yin et al., 2022)

2.3 Meditation, Stress Regulation and Meditative Movement

One limitation of the current exercise-microbiome literature is that it often emphasizes energy expenditure and training load while giving less attention to psychological regulation. This matters because stress is a major determinant of gut microbial composition, intestinal permeability, immune activation, and gut-brain signaling. Prolonged psychological stress can reduce microbial diversity, promote dysbiosis, and contribute to both gastrointestinal and affective symptoms (Beurel, 2024). If stress shapes the gut environment, then movement practices that reduce stress may influence the microbiota through mechanisms that differ from

those of purely performance-driven exercise.

Meditation is widely acknowledged for its ability to reduce stress, improve emotional regulation, reduce inflammation, and modulate neuroendocrine pathways (Sparacio et al., 2024). Mindfulness-based practices reduce cortisol levels, improve cardiovascular control, and reduce systemic inflammatory markers (Hooi et al.; Philip et al., 2025). Stress is a fundamental regulator of gut microbial composition and intestinal permeability, and prolonged psychological stress alters microbial diversity and promotes dysbiosis, which contributes to gastrointestinal and mood problems. Meditation and mindfulness, on the other hand, may help to maintain a more stable gut environment by reducing inflammatory cytokine signalling and modulating vagal tone (Bhadoriya et al., 2025). These findings lend support to the concept that practices that combine physical activity and meditative involvement may have a synergistic effect on both mental and gastrointestinal health (Yao et al., 2025).

Meditative movement practices are especially interesting because they integrate physical activity with attentional regulation, breathing control, and embodied awareness. Traditional martial arts, yoga, qigong, and related disciplines are not solely exercise modalities; they are also regulatory practices that may act simultaneously on physical, emotional, and neuroendocrine systems (Grimonprez et al., 2015). Such practices may influence the gut-brain-microbiota axis through multiple overlapping routes: moderate movement, reduced psychological stress, altered inflammatory signaling, enhanced parasympathetic activity, and improved emotional self-regulation. This multidimensional profile suggests that meditative movement may offer a distinct model for microbiome research, one that is not captured adequately by studies of competitive sport alone.

2.3.1 Tai Chi and Aikido as Contemplative Martial Arts

Martial arts have a history that spans thousands of years and are generally recognized as systems of combat, both offensive and defensive. These practices may involve unarmed techniques or the use of weapons. Many modern martial arts trace their origins to the East and have evolved into disciplines focused on sport, self-defense, and recreation. Traditional martial arts schools often include mental or meditative elements in their training to promote personal growth and positive transformation. Practices that combine deliberate movement, breath control, and prolonged mental focus, also known as meditative movement, may affect physiological and psychological systems at the same time, influencing the gut-brain-microbiota axis via separate processes (Grimonprez et al., 2015). Physical exercise also has an impact on the autonomic nervous system, namely vagal nerve function, a critical component of the gut-

brain axis whose dysfunction is intimately associated to depression (Browning & Travagli, 2019). Martial arts are a distinct set of mind-body practices that combine physical activity, breath regulation, focus, and philosophical belief (Pujari, 2021). Tai Chi and Aikido, both steeped in Eastern philosophy, are soft martial arts that emphasize synchronized movement, balance training, controlled breathing, and increased body awareness (Jennings & Delamont., 2023). These practices prioritize harmony, proprioception, and mental clarity, establishing them as archetypal forms of contemplative movement rather than performance-driven exercise (Liu et al., 2025). Martial arts practice has been linked to improvements in physical fitness, emotional regulation, psychological well-being, and social connectedness, as well as reductions in anxiety and depressive symptoms, particularly in non-clinical, community-based populations (Guo et al., 2016).

Within the broad category of meditative movement, Tai Chi and Aikido are particularly relevant. Both are often described as soft martial arts rooted in Eastern philosophical traditions and characterized by coordinated movement, balance, breathing, concentration, and heightened body awareness (Jennings & Delamont., 2023). Unlike high-intensity or combat-oriented training systems, these practices emphasize control, harmony, and internal regulation rather than maximal output or competition. As a result, they may be especially suitable for examining how moderate movement combined with mindfulness affects physiological systems linked to the gut microbiota.

Tai Chi is a low-impact internal martial art distinguished by gentle, flowing movements and sustained meditative awareness, with research indicating advantages for mental health, balance, cardiovascular function, and immune-inflammatory control (Yang et al., 2025). The available literature also indicates that Tai Chi and other martial arts may reduce depressive symptoms, particularly in the short term, with mental health benefits partially mediated by social connectedness arising from group-based practice settings, as demonstrated by mediation analyses in intervention studies. Aikido is a distinct Japanese meditative martial technique that emphasizes harmony, circular and flowing motions, breath regulation, and embodied awareness (Lukoff & Strozzi-Heckler. , 2017). Aikido, sometimes known as "meditation in action," promotes increased attention, focus, and psychological quiet during practice (Lothes et al., 2013). Although less well-studied than Tai Chi, Aikido shares key features of mind-body integration and may have similar effects on mental and physiological pathways, such as stress-responsive and immune-related systems (Yin et al., 2023). The metabolic intensity of martial arts varies greatly: Tai Chi ranges from 1.5 to 3.0 METs (Metabolic Equivalent of Task) (light-to-moderate intensity), slower-paced Aikido averages around 5.3 METs (moderate intensity),

and combat sports like judo, karate, and kickboxing reach 10.3 METs (vigorous intensity) (Ainsworth et al., 2011). Unlike high-intensity competitive sports, contemplative martial arts combine moderate physical activity with mindfulness and self-regulation, potentially providing distinct advantages for mental health (Prakhinkit et al., 2014). They comply with international physical activity guidelines by functioning at light-to-moderate intensity levels and may represent sustainable, accessible types of movement throughout the life span (Liu et al., 2025). Nonetheless, actual research on gut microbial profiles in individuals practicing contemplative martial arts is scarce. While substantial research has been conducted on the gut microbiota of competitive athletes, revealing sport-specific microbial changes influenced by training intensity and physiological load, non-competitive, contemplative martial arts remain understudied. Studies show that the type of sport, intensity of training, and physiological load all influence gut microbial profiles, with endurance, strength, and combat sports having distinct signatures (Ghaffar et al., 2024). Both practices have been shown to reduce stress and improve overall health. Tai Chi, for example, has been associated with improved immune function, reduced inflammation, and better cardiovascular health. Aikido, while less studied in this regard, has similarly been linked to mental well-being and reduced anxiety. Both practices focus not only on physical movement but also on meditation, making them ideal candidates for studying the effects of mind-body practices on gut health. However, much prior research has focused on competing athletes participating in high-intensity or performance-driven activities, leaving a significant gap in understanding non-competition, contemplative martial arts such as Tai Chi and Aikido.

3 RESEARCH GAP AND RATIONALE

Despite the growing literature on exercise and the gut microbiota, an important gap remains. Most available research has examined competitive athletes, endurance training, resistance exercise, military training, or other high-load, performance-oriented contexts. These studies have generated valuable insight into how exercise type, intensity, physiological stress, and diet shape the gut microbial environment. However, they do not fully address whether moderate, contemplative, and non-competitive movement practices may influence microbiota through partly different mechanisms. This is a notable omission because mind-body practices combine physical activity with stress regulation, breath control, attentional focus, and often social engagement.

The current thesis is grounded in the view that these features may matter biologically. Tai Chi

and Aikido may influence the gut not only through movement itself, but also through reduction of psychological stress, improved autonomic balance, altered inflammatory signaling, and broader gut-brain-muscle communication. Existing evidence already suggests that both practices are associated with improvements in mental well-being and broader health outcomes, yet direct investigation of gut microbial profiles in practitioners of contemplative martial arts remains scarce (Ghaffar et al., 2024). As a result, current knowledge remains weighted toward athlete populations and may not generalize meditative movement practices. Addressing this gap is important for both theoretical and practical reasons. Theoretically, studying contemplative martial arts may refine understanding of how exercise influences microbiota by highlighting the potential role of psychological regulation and autonomic balance alongside physical workload. Practically, such work may have broader public health relevance than studies limited to elite athletes, because practices like Tai Chi and Aikido are more accessible and sustainable for diverse populations. If they are associated with favorable microbial patterns or related health outcomes, they may offer an additional lifestyle-based route for supporting gastrointestinal, metabolic, and psychological well-being.

3.1 Aim and Objective of the Study

The overall aim of this thesis is to examine the relationship between contemplative martial arts practice and the gut microbiota, with a particular focus on Tai Chi and Aikido as mind-body movement disciplines. The thesis seeks to situate these practices within the wider literature on microbiota, exercise, and brain-gut-muscle communication, and to clarify why they may represent an important but underexplored area of inquiry.

The specific objectives are:

1. To outline the biological importance of gut microbiota in human health and disease.
2. To review the evidence linking physical activity and exercise with gut microbial diversity, composition, and metabolite production.
3. To examine how exercise type, intensity, physiological load, and meditative practices shape microbial outcomes in athletes and physically active populations
4. To discuss the gut-muscle axis and the broader brain-gut-muscle axis as frameworks for understanding interactions among exercise, metabolism, and microbiota.
5. To evaluate the relevance of meditation, stress regulation, and autonomic function in relation to gut microbial health.
6. To explore Tai Chi and Aikido as contemplative martial arts that may influence the gut microbiota through both physical and psychophysiological pathways.

7. To identify the current research gap and justify the importance of further investigation into contemplative martial arts and gut health.
8. To perform genome-resolved metagenomic analysis by preparing MAGs from publicly available shotgun metagenomic datasets representing different sport modalities and sedentary controls.
9. To evaluate whether MAG-based profiling can provide higher-resolution insight into exercise-intensity-associated microbial signatures and support a more precise interpretation of microbiota adaptation in physically active populations.

3.2 Significance of the Study

This study contributes to an emerging interdisciplinary field at the intersection of microbiome science, exercise physiology, and mind-body medicine. Its significance lies in challenging the assumption that exercise-related microbial change is driven only by training intensity or athletic performance. By focusing on Tai Chi and Aikido, the study broadens the conceptual scope of exercise-microbiome research and draws attention to the potential relevance of breathing, attentional focus, stress reduction, and embodied awareness.

The thesis is also significant because it centers forms of movement that may be more feasible for wider populations than vigorous sport. If contemplative martial arts prove relevant to gut microbial health, their value will extend beyond specialist or elite groups and into preventive and lifestyle-oriented health contexts. This has implications for future research on accessible non-pharmacological strategies that support mental, metabolic, and gastrointestinal well-being.

Furthermore, the inclusion of metagenome-assembled genomes adds a genome-resolved perspective to the thesis by complementing 16S rRNA-based microbiota profiling. This approach provides a more detailed understanding of microbial signatures associated with physical activity and exercise intensity, thereby strengthening the interpretation of microbiota changes in relation to health and lifestyle-based interventions.

4 MATERIALS AND METHODS

4.1 REVIEW OF THE LITERATURE

A first literature review focusing on gut microbiota in different physical activities was conducted to investigate how gut microbial composition is modulated by various types and intensities of exercise and by the diverse lifestyles of athletes. Building on this, a second review examined the effects of intermittent fasting on the physical performance of athletes in different

sports, with particular attention to how performance can be optimised while following intermittent fasting regimens, thus integrating nutritional and training-related factors. Finally, a third review aimed to evaluate and synthesise current knowledge on sex-related differences in gut microbiota across human and animal studies, providing a broader biological context for interpreting microbiota variability between individuals.

4.1.1 Systematic review and metanalysis: Gut microbiota role in different physical activities

4.1.1.1 Selection protocol and search strategy

The systematic review was conducted following PRISMA guidelines (Page et al., 2021) and registered in PROSPERO (CRD42024500826). We applied a PICOS framework with the following criteria: (a) healthy human populations; (b) any type of physical activity as the intervention; (c) comparison with no training or other types of physical conditioning; (d) outcomes assessing gut microbiota through α- and β-diversity, relative abundance of specific taxa and/or metagenomic data analysed by 16S rRNA sequencing; (e) articles written in English; and (f) observational (cross-sectional, retrospective, prospective) or experimental study designs. We excluded clinical trials, reviews, meta-analyses, case reports, qualitative studies, conference proceedings, commentaries, studies without a control group, those involving unhealthy subjects, and incomplete designs. PubMed, ScienceDirect and Scopus were searched up to April 2023 using combinations of terms related to “gut microbiota”, “physical activity”, “diet” and “microbiome”, with MeSH terms applied in PubMed and title/abstract/keyword searches in Scopus and Web of Science. Titles and abstracts retrieved from all databases were imported into Covidence for screening, and four experts independently reviewed studies in multiple steps to assess eligibility.

4.1.1.2 Data extraction process and quality assessment

From each eligible study, we extracted bibliographic details, study design, population characteristics, type of exercise, duration, frequency and training volume, as well as information on microbial profiling. Study quality was assessed using tools appropriate to each design. Cross-sectional studies were evaluated with the Newcastle Ottawa Scale ("The Newcastle-Ottawa Scale (Nos) for Assessing the Quality of Non-randomised Studies in Meta-Analyses," 2021) adapted for cross-sectional designs, and cohort studies with the Newcastle Ottawa Scale for Cohort Studies (Moher et al., 2009). Articles were then classified as good, fair, or poor quality based on the number of criteria met, reflecting low, moderate or high risk of bias, respectively. Randomized clinical trials were assessed with the Cochrane RoB2 tool, considering five domains (randomization, deviations from intended interventions, missing

data, outcome measurement and selective reporting) to assign an overall risk of bias rating (Sterne et al., 2019).

4.1.1.3 Data synthesis

For differences in overall gut microbiota composition, we performed only a descriptive synthesis because of heterogeneity in how microbial proportions were reported, small sample sizes, and limited data quality across studies. Quantitative synthesis was conducted using Comprehensive Meta-Analysis (Cleophas & Zwinderman, 2007), focusing in particular on the Firmicutes/Bacteroidetes (B/B) ratio, a commonly used index of gut health. When unit-of-analysis issues were present, only the total number of participants in the control group was adjusted, while means and standard deviations were left unchanged. Standardized mean differences (SMDs) with 95% confidence intervals (95% CI) were calculated, and effect sizes were interpreted as small (SMD = 0.2), moderate (SMD = 0.5), and large (SMD = 0.8) (Higgins et al., 2003). Between-study heterogeneity was assessed using Cochran's Q (Hedges' Q statistic) and the I^2 index, with thresholds of <25% indicating low, <50% moderate, and >75% high heterogeneity (Bender et al., 2018). Publication bias was explored using Egger's test and funnel plots. Subgroup analyses were conducted for outcomes reported in at least two studies per subgroup, and meta-regression and subgroup analysis were used to investigate potential sources of heterogeneity. Predefined subgroups included chronic versus acute interventions, aerobic versus anaerobic exercise, physical activity intensity, sample size, age, publication year, and methodological quality. Exercise intensity was classified using percentages of maximal aerobic power (VO_2max) and metabolic equivalent (MET) values (Ainsworth et al., 2011; Mitchell et al., 2005). Sensitivity analyses were performed by excluding individual studies or modifying the effect model.

4.1.2 Narrative review: Impact of intermittent fasting on physical performance in different sports

Considering the findings from the first systematic review, which highlighted the complex interplay between exercise, gut microbiota and lifestyle factors, a second, narrative review was conducted to specifically address how intermittent fasting, in the form of Ramadan fasting, influences athletes' physical performance and related health outcomes

4.1.2.1 Search strategy

The review systematically searched PubMed, Google Scholar and Scopus for English-language articles published between 2014 and 2024 that investigated the effects of Ramadan fasting on athletes physical performance. The review question was structured according to the PICOS framework with the following criteria: (a) Population: athletes; (b) Intervention: Ramadan

fasting; (c) Comparison: age- and condition-matched control groups; (d) Outcomes: measures of physical performance and health; and (e) Study type: all empirical study designs. The search strategy combined terms related to “Ramadan fasting” and “physical activity/sport”, restricted to the specified time frame and language. Only studies specifically examining the impact of Ramadan fasting on different categories of athletes were included, whereas studies focusing on fasting in non-athlete or generally active healthy populations were excluded.

4.1.2.2 Data extraction & inclusion exclusion criteria

After screening titles and abstracts, the eligible studies were classified into three categories including individual sports, team sports and combat sports. For this review, we included randomized studies published between 2013 and 2024 that examined the impact of Ramadan intermittent fasting on nutrition and physical performance in healthy athletes (17–40 years of age) from developed and Muslim countries, and that reported outcomes in specific sports disciplines. Eligible studies had to assess at least one of the following: anthropometric or nutritional changes (e.g. BMI, body composition, biochemical markers, dietary intake), physical performance (e.g. VO_2max, fatigue, agility, sport-specific performance) or behavioural adaptations related to sport strategies. We focused on recent, peer-reviewed articles directly addressing the effects of Ramadan fasting or related Muslim religious dietary practices on athletes’ physiological health. Studies involving non-athletes, sedentary individuals, or lacking relevant fasting or performance outcomes were excluded. All search results were exported to an EndNote library; removed duplicates and screened titles and abstracts against the inclusion criteria, recording reasons for exclusion e.g. wrong population, no Ramadan intermittent fasting, inappropriate study type or outcomes before proceeding to full-text assessment of potentially eligible articles.

4.1.3 Systematic review: Sex related differences in gut microbiota

Following the reviews on exercise- and fasting-related modulation of gut microbiota, a third systematic review focused on sex-related differences to account for an additional layer of host variability. By comparing gut microbial diversity and composition between males and females in both humans and animals, this review provides a broader biological context for interpreting the heterogeneous microbiota responses observed in relation to physical activity and dietary patterns

4.1.3.1 Selection protocol and search strategy

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Rethlefsen et al., 2021). The

protocol was registered in PROSPERO (CRD420251020857), and the methods were developed following the Cochrane Handbook for Systematic Reviews of Interventions. A systematic search of PubMed, Scopus, ScienceDirect and Google Scholar identified English-language articles published between 2006 and 2024 investigating sex-related differences in gut microbial diversity in humans and animals. The review question was structured using the PICOS framework with the following eligibility criteria: (a) population: healthy subjects; (b) intervention: none (observational studies only); (c) comparison: age- and sex-matched control groups; (d) outcomes: sex differences in gut microbiota, including α- and β-diversity, relative abundance of specific taxa and/or metagenomic data analysed by 16S rRNA amplicon or shotgun sequencing; (e) language: English; and (f) study design: observational (cross-sectional, cohort, case–control, nested case–control) or experimental studies. Studies involving unhealthy participants, lacking appropriate control groups, using purely culture-based methods, or focusing on non-intestinal microbiota (e.g. skin, oral, gastric, nasopharyngeal) were excluded, as were clinical trials, reviews, meta-analyses, case reports, conference proceedings, qualitative and commentary articles. Search strategies combined terms related to “gut microbiota”, “sex”, “gender” and “microbiome”, using title, abstract and MeSH term searches in PubMed and title/abstract/keyword searches in Scopus and Web of Science. Search results were imported into a reference manager, and study selection followed a multi-step screening process in which four reviewers independently examined titles and abstracts against the inclusion criteria, with reasons for exclusion (e.g. wrong population, no sex comparison, non-gut microbiota, inappropriate design or outcomes) recorded before full-text assessment.

4.1.3.2 Data analysis

After screening titles and abstracts, the eligible studies were classified into two categories based on their main characteristics. One category includes human studies regarding sex differences in gut microbiota and second category includes animal studies regarding sex differences in gut microbiota.

4.1.3.3 Data synthesis and inclusion exclusion criteria

Inclusion criteria for this review were: (1) studies published in the last 20 years that evaluated differences in gut microbial diversity according to sex; (2) studies investigating gut microbial communities in male and female human or animal populations; and (3) studies reporting sex-related differences in gut microbiota. Only studies involving healthy subjects were included, without specific age restrictions, to capture a broad range of data. Outcomes considered included at least one of the following: anthropometric measures, intestinal microbiota markers, dietary intake, or sex hormone levels. Studies were excluded if they did not analyse sex-based

differences or involved unhealthy populations. All records were imported into an EndNote library for the selection phase. The primary author removed duplicates and screened titles and abstracts against the inclusion criteria, documenting reasons for exclusion (e.g. not an original study, incorrect population, not a sex-based analysis, or irrelevant outcomes). Given the heterogeneity in how microbial proportions were reported, small sample sizes, insufficient data, frequent absence of control groups and variable study quality, a descriptive synthesis of the literature was carried out rather than a formal meta-analysis.

4.2 REGULATION OF GUT MICROBIOTA IN AIKIDO ATHLETES

4.2.1 Ethical approval and study design

The study protocol was reviewed and approved by the Institutional Review Board of the University of Rome "Foro Italico" (approval No. 204/2024). Recruitment took place among Aikido practitioners after their regular training sessions. Before enrolment, the aims of the study, the experimental procedures, and the associated ethical safeguards (including anonymity, confidentiality, and voluntary participation) were explained in detail. All participants provided written informed consent prior to inclusion, in accordance with the principles of the Declaration of Helsinki. This study involved adult Aikido practitioners regularly attending scheduled classes. Each participant received a faecal swab kit (Copan Italia S.P.A., Brescia, Italy) together with written instructions for sample collection at home. Participants were asked to collect the faecal sample and deliver the swab on a pre-arranged day within two hours of collection. Upon delivery, samples were kept at 4–8 °C in a refrigerated container and transported within 24 hours to the laboratory of the University of Rome "Foro Italico", where they were processed using a previously validated protocol for DNA extraction from faecal traces. Salivary swabs were collected and handled according to the same storage and transport conditions. In total, 28 Aikido practitioners were invited to take part in the study and were provided with standardized sampling kits and detailed instructions. Of these, 25 agreed to participate and completed all required procedures, including signing the informed consent form, filling in the questionnaire, and providing both biological samples. Two participants were subsequently excluded from the final analysis due to non-compliance with the protocol (e.g. incorrect labelling or delayed sample delivery). The final dataset therefore comprised 23 participants with complete and analysable data (Fig 5). Inclusion criteria were regular practice of Aikido, willingness to provide faecal and salivary samples, completion of the survey, and absence of antibiotic or probiotic use or gastrointestinal procedures in the three months prior to enrolment. All data were anonymized by assigning each participant a unique

code generated from non-identifiable information, which was used to match questionnaire responses with biological samples.

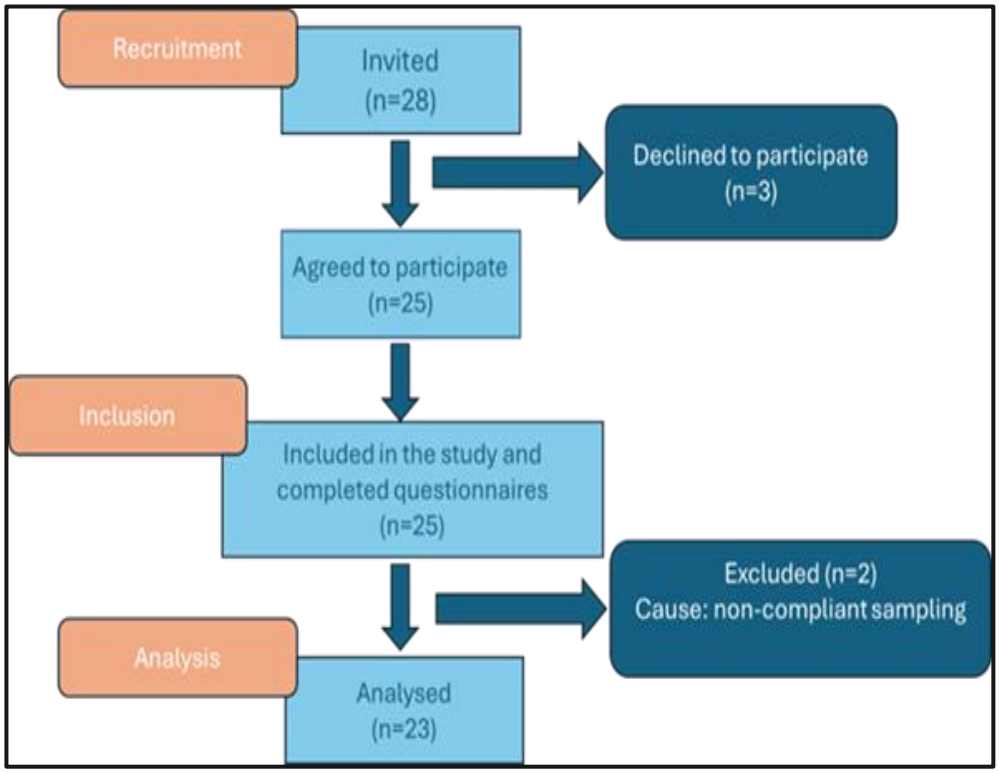


Figure. 5 Flowchart of the study**,** Recruitment, inclusion, and analysis phases of participants enrolled in the Aikido

4.2.2 Questionnaires

Participants completed an online questionnaire administered via the EU Survey platform, which collected information on age, sex, weight, height and specific dietary patterns (e.g. vegetarian or vegan). These variables were recorded for descriptive purposes only and did not constitute exclusion criteria. Additional questions assessed the presence of chronic diseases, pregnancy, food intolerances, concomitant infections, recent use of antibiotics or probiotics, and gastrointestinal surgical procedures in the previous three months; any of these conditions led to exclusion from the study. Dietary habits and habitual physical activity levels were evaluated using the 9-item questionnaire developed by Martínez-González et al. and the short form of the International Physical Activity Questionnaire (IPAQ), respectively (Martínez-González et al., 2012; Meh et al., 2021). To assess meditation practice, participants also completed the 35-item Meditation Depth Questionnaire (MEDEQ), developed together with the Meditation Depth Index (MEDI) by Piron(Piron, 2022).The first part of the MEDEQ gathers information on type of meditation, years of practice and average duration of each session, whereas the second part consists of 30 items describing physical and mental sensations experienced during meditation. For each item, participants indicated their level of agreement

on a 0–4 Likert scale (0 = no agreement, 4 = strong agreement). The MEDI score, representing the depth of meditation, was calculated according to the original scoring scheme by summing items that are positively associated with meditative experience (factors 3, 4, 6, 7, 8, 9, 11, 12, 15, 16) and reverse-scoring items that are negatively associated with meditation (factors 16, 17, 19–30 and items 1, 4, 9, 12, 13 and 18). Thus, higher MEDI values reflect a deeper subjective meditative experience and are independent of the time spent meditating, focusing instead on the quality of physical and mental sensations during practice.

4.2.3 Data Collection

The sample size (n = 23) provided 84% power to detect a difference corresponding to an effect size of 0.7 at an alpha level of 0.05, using paired two-tailed t-tests (Nieman & Wentz, 2019). Data collection was carried out after a brief presentation of the study at the end of Aikido classes. Participants who agreed to take part received a standardized kit on pre-arranged days. The kit included an information sheet, informed consent form, written instructions, a tube for faecal sampling, a tube for salivary sampling, adhesive labels for tube identification and a link to the online questionnaire. Faecal and salivary samples were collected independently at home following the written instructions, stored in a refrigerator until delivery, and returned to the researchers on scheduled days and times. To preserve anonymity, each participant generated an identification code composed of the first three letters of the mother's first name and the father's year of birth (e.g. FRA1967). This code, known only to the participant, was written on the labels affixed to both sampling tubes and entered into the questionnaire, thereby enabling linkage of biological and questionnaire data without revealing personal identities. The questionnaire, which included both closed- and open-ended items, was administered via a secure link using the EU Survey platform, the official European Commission tool for managing online surveys.

4.2.4 DNA Extraction and Illumina Sequencing

A faecal swab (Copan Italia S.P.A., Brescia, Italy) was supplied to each participant together with written instructions for stool collection. Participants were asked to collect the sample at home and return it on a pre-arranged date, within two hours of defecation. On delivery, samples were stored at 4–8 °C in a refrigerated container and transported within 24 hours to the laboratory of the University of Rome "Foro Italico". In the laboratory, faecal material was weighed, and microbial DNA was isolated using a previously validated protocol for DNA extraction from faecal traces(Valeriani et al., 2018). The extracted DNA was subsequently purified and normalised before library preparation.

Amplicon libraries targeting the V1–V3 hypervariable regions of the 16S rRNA gene were generated according to the 16S Metagenomic Sequencing Library Preparation Guide (part #15044223 Rev. A; Illumina, San Diego, CA, USA). A two-step tagged PCR strategy was adopted: in the first reaction, adapter-containing primers specific for the 16S rDNA region were used to amplify the target, and in the second reaction, barcode-containing primers were applied to index individual samples. Each PCR reaction (25 µL) contained 12.5 µL KAPA HiFi HotStart Ready Mix (Roche, Pleasanton, CA, USA), 5 µL of each primer (1 µM) and 2 µL of template DNA. Amplifications were performed on a Techne® TC-PLUS thermal cycler (VWR International, LLC, Radnor, PA, USA). Successful amplification was verified by loading 5 µL of PCR product on a 1% agarose gel. Clean amplicons were quantified using the Qubit PicoGreen dsDNA BR Assay Kit (Invitrogen, Grand Island, NY, USA). Libraries were then prepared following the MiSeq Reagent Kit Preparation Guide (Illumina) and sequenced on the iSeq 100 platform (Illumina, San Diego, CA, USA). Raw sequencing reads were processed using a customised bioinformatic workflow implemented on the Galaxy platform, combining quality-control and taxonomic-assignment tools. Initial inspection of FASTQ files was performed with FASTA/Q information tools and Mothur. Reads shorter than 200 nucleotides, containing ambiguous bases or identified as chimeric were removed. High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity using a de novo clustering approach. Representative sequences from each OTU were taxonomically assigned with the Ribosomal Database Project (RDP) Classifier (version 2.5), based on a naïve Bayesian method. OTUs at 97% similarity were treated as species-level units, whereas 95% similarity thresholds were used for genus-level assignments. In parallel, sequences were analysed with the Illumina BaseSpace 16S Metagenomics App (version 1.0.1), which applies an independent taxonomic classification pipeline using the curated May 2013 release of the Greengenes reference database (Colwell et al., 2012). Only taxa with a relative abundance greater than 0.1% were retained for downstream statistical analyses.

4.2.5 Statistical Analysis

A descriptive statistical analysis was performed on all collected variables. Body mass index (BMI), used as an anthropometric indicator, was calculated as body weight (kg) divided by the square of height (m^2). Cohort characteristics (age, BMI, diet type and habitual physical activity level) are reported as mean ± standard deviation (SD) and range. Participants were also classified according to their meditation score into three categories: low (MED 0, 0–60 points; n = 11, 44%), medium (MED 1, 60–80 points; n = 7, 28%) and high (MED 2, 80–120 points;

n = 7, 28%), and according to their level of Aikido practice. The meditation score reflects the intensity of physical and mental sensations experienced during practice rather than time spent meditating. Relative abundances of gut microbial taxa were calculated at different taxonomic ranks. The proportion of the microbiome assigned to each phylum, class, order, family and genus was estimated using the RDP Classifier and the Greengenes reference database. Briefly, relative abundance for each phylotype was computed as: % abundance = (number of sequences assigned to a given phylum or genus / total sequences per sample) × 100. For downstream analyses, only the more prevalent phyla were retained; the 24 rarest phyla, defined as those with relative abundance <0.1% across all sam ples, were excluded. Alpha and beta diversity indices were calculated in PRIMER 7 at 97% sequence similarity (Magurran, 2021).Alpha diversity was evaluated using the Shannon diversity index and a species-level evenness index. Differences in community structure between groups were explored by principal coordinate analysis (PCoA) based on beta-diversity distance matrices (Gallè et al., 2020). Student's t-tests were applied to compare bacterial diversity measures and the relative abundance of dominant phyla and genera (constituting approximately 90% of the faecal bacterial community) between groups defined by Aikido level and meditation category. Analysis of variance (ANOVA) with Bonferroni post hoc correction was used to test differences among groups stratified by habitual physical activity levels and among lifestyle subgroups combining meditation depth and physical activity. The statistical significance of factors potentially contributing to overall compositional differences between microbiota profiles was further assessed in PRIMER 7 using the non-parametric ANOSIM (analysis of similarity) procedure with default settings. To identify determinants of microbiome composition, two linear regression models were built with age, sex, BMI, diet and Aikido level as independent variables and either the Shannon index or the Firmicutes/Bacteroidetes (F/B) ratio as dependent variables. A stepwise approach was used to account for potential confounders. A p-value < 0.05 was considered statistically significant. All statistical analyses (except diversity and ANOSIM analyses) were performed using IBM SPSS Statistics, version 25 (IBM Corp., Armonk, NY, USA).

4.3 ASSOCIATIONS BETWEEN MOVEMENT-MEDITATION PRACTICES AND INTESTINAL MICROBIOTA IN TAICHI & AIKIDO ATHLETES

4.3.1 Subject enrolment & sample data collection

The study protocol was approved by the Institutional Review Board of the University of Rome "Foro Italico" (approval no. CAR 204/2024) and was conducted in accordance with the

principles of the Declaration of Helsinki. During recruitment, the study aims, procedures, and ethical safeguards including anonymity, confidentiality, and secure handling of data were clearly explained to prospective participants. Written informed consent was obtained from all participants prior to enrolment. Fifty-three adult Tai Chi and Aikido practitioners were invited to participate following their routine training sessions. The Aikido participants had previously been enrolled in Study 1 of this thesis, and their microbiota data were collected as part of that earlier investigation. Their inclusion here constitutes a secondary use of an existing dataset for the purpose of cross-disciplinary comparison. Ethical approval for the original Aikido data collection was granted under the same protocol as Study 1 (approval No. 204/2024). The Tai Chi participants were recruited de novo for this study. The two cohorts are mutually exclusive participant appears in both groups andwere treated as independent samples in all analyses. Of these, 46 consented and completed all study procedures, including completion of questionnaires and provision of biological samples. Four samples were subsequently excluded from the final dataset due to protocol non-compliance (e.g., incorrect labelling or delayed submission). The final analytical cohort therefore comprised 42 participants as shown in Figure 6.

Eligibility criteria included regular practice of Tai Chi (Tai Chi group) or Aikido (Aikido group), willingness to provide biological samples and complete questionnaires, and no use of antibiotics or probiotics or gastrointestinal procedures within the preceding three months. Participants were adults (≥18 years, including individuals >65 years), with sex distribution. To protect privacy, all participant information was anonymized using a unique, non-identifiable alphanumeric code applied consistently to both questionnaires and biological samples. All inclusion and exclusion criteria including recent antibiotic/probiotic use, recent gastrointestinal procedures, chronic diseases, pregnancy, and other medical conditions with potential to influence gut microbiota composition were assessed during recruitment, prior to enrolment and sample collection.

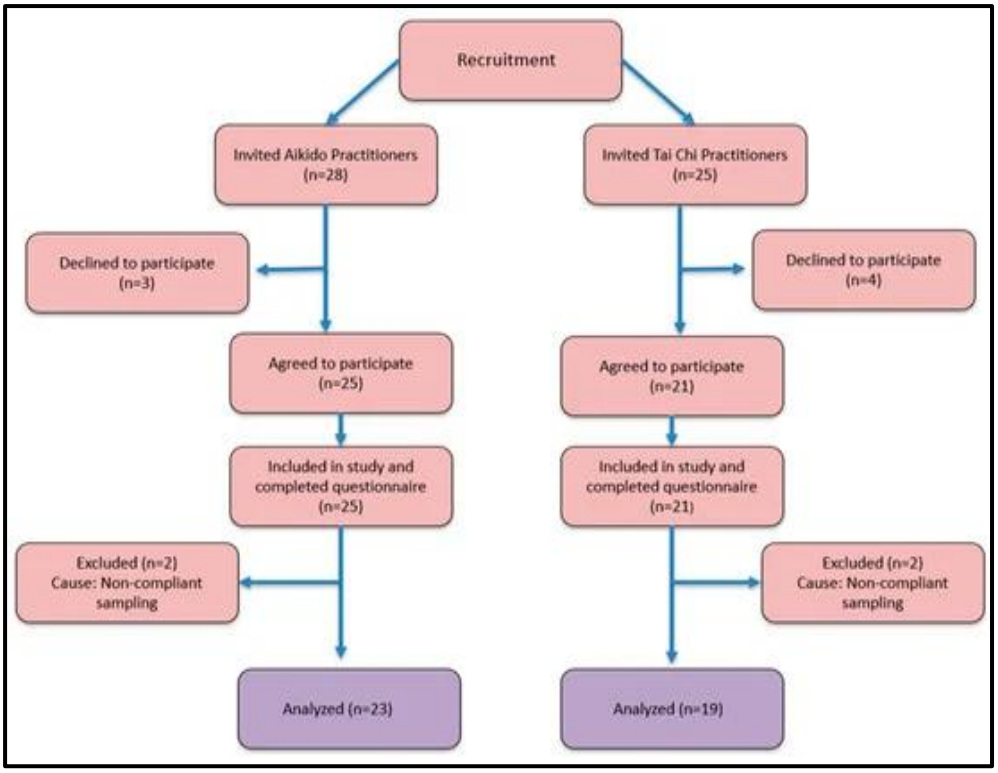


Figure. 6 Flowchart of the study**,** Recruitment, inclusion, and analysis phases of participants

4.3.2 Biological Sample Collection and Handling

Each participant was provided with a fecal swab (Copan Italia S.p.A., Brescia, Italy) and standardized instructions for sample collection. In accordance with the cross-sectional study design, one fecal sample was obtained from each participant. Participants were instructed to collect the sample on a designated day and to return the swab within 2 h of collection. Although a strict circadian sampling window was not enforced, collection was requested under consistent daily conditions, and participants were advised to avoid sampling immediately after intense physical activity. Following collection, samples were maintained at 4–8 °C in a refrigerated container and transported to the laboratory at the University of Rome “Foro Italico” within 24 h, where they were processed using a previously validated protocol for DNA extraction from fecal material.

4.3.3 Questionnaires

Participants were asked to fill out a questionnaire on the EU survey platform, which collected details about their age, gender, weight, height, and any specific dietary practices they might follow (such as vegetarian or vegan diets). It was emphasized that providing this information would not result in the exclusion of any participants. Furthermore, the presence of ongoing chronic illnesses, pregnancy, food sensitivities, concurrent infections (along with the use of antibiotics and probiotics), or gastrointestinal surgical procedures within the last three months

were considered as exclusion criteria. The 9-item questionnaire created by Martínez-González et al. and the abbreviated version of the International Physical Activity Questionnaire (IPAQ) were utilized to evaluate participants' eating habits and typical physical activity levels (Martínez-González et al., 2012; Meh et al., 2021). In addition, a 35-item evaluation tool known as the Meditation Depth Questionnaire (MEDEQ) was implemented to assess the intensity of meditation engaged in by each participant (Piron, 2022). The opening questions of this questionnaire focus on the techniques and duration of meditation, covering both the number of years practiced and the minutes spent in each session. The latter section consists of 30 items aimed at gathering insights about the physical and mental experiences encountered by subjects during meditation. This information is then leveraged to calculate the MEDI, a quantitative metric for meditation depth. For every question, participants were asked to indicate their degree of agreement on a scale from 0 (indicating no agreement) to 4 (indicating strong agreement). The overall score was derived by adding up the factors identified as supportive of meditation specifically factors 3, 4, 6, 7, 8, 9, 11, 12, 15, 16. The final score is reached by summing up the scores for factors 16, 17, 19-30, and items 1, 4, 9, 12, 13, and 18, which are inversely linked to meditation. It is important to highlight that the total score does not pertain to the time element, but rather to the physical and mental sensations experienced during meditation. In the present study, these instruments were therefore employed as exploratory measures to characterize relative differences in meditation depth and overall physical activity levels within the study cohort, rather than for diagnostic use or for comparative analyses across populations.

4.3.4 Data Collection

Given the exploratory, cross-sectional design, no formal a priori sample size calculation was undertaken to power predefined hypothesis-testing comparisons. The final sample size was therefore determined by feasibility and participant availability during the recruitment period. Any previously reported power considerations based on paired designs were removed, as they were not applicable to the present unpaired study. Participants were recruited following an invitation delivered at the end of training sessions. Individuals who agreed to participate received the study materials on pre-scheduled days, including an information sheet, informed consent form, written instructions, fecal collection tubes with corresponding labels, and a link to the study questionnaire. Fecal samples were collected separately in accordance with detailed instructions and were stored under refrigeration until delivery on designated days and time windows. To ensure anonymity, each participant was assigned a non-identifiable code

generated from the first three letters of the mother's name and the father's birth year (e.g., FRA1967), which was not accessible to the research team. This code was applied to the sample tube via the provided labels and entered in the questionnaire, enabling linkage of questionnaire data and biological samples for integrated analyses. The questionnaire comprised both open-ended and closed-ended items and was administered via the EU Survey platform, the European Commission's official tool for online survey deployment.

4.3.5 DNA Extraction from 16S rDNA Amplicon

A faecal swab (Copan Italia S.P.A., Brescia, Italy) was given to each participant, along with detailed instructions for stool sample collection. Participants were told to submit their samples on a specified date, within two hours of collection. The samples were kept at 4–8 °C in a refrigerated container and transported to the laboratory at the University of Rome "Foro Italico" within 24 hours. Upon receipt, faecal samples were weighed, and DNA was extracted following a validated protocol for isolating microbial DNA from faecal material. The DNA was then purified and normalized before sequencing. Amplicon libraries were created targeting the V1–V3 region of the 16S rRNA gene, adhering to the 16S Metagenomic Sequencing Library Preparation Guide (part# 15044223 Rev. A; Illumina, San Diego, CA, USA)(Valeriani et al., 2018b). Tagged polymerase chain re-action (PCR) products were generated through a two-step PCR process using unique barcoded primer pairs. In this approach, the target primers containing adapters were employed in the first PCR reaction to amplify the target gene (16S rDNA), and the resulting product was used in the second PCR with primers that included barcodes for individual sample identification. Each amplification reaction comprised a total volume of 25 µL, including 12.5 µL of KAPA HiFi Hot Start Ready Mix (Roche, Pleasanton, CA, USA), 5 µL of each primer (1 µM), and 2 µL of template deoxyribonucleic acid (DNA). Reactions were conducted on a Techne® TC-PLUS thermal cycler (VWR International, LLC, Radnor, PA, USA).

4.3.6 Illumina Sequencing and Bioinformatics Analysis

The amplification is performed and 5 µL of PCR product from every reaction was analysed through agarose gel electrophoresis (1%) to verify amplification. The final concentration of the purified DNA amplicon was measured using the Qubit PicoGreen dsDNA BR Assay Kit (Invitrogen, Grand Island, NY, USA). Libraries were prepared following the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) and utilized the iSeq100 sequencing platform (Illumina, San Diego, CA, USA). Raw sequencing data were processed with a customized bioinformatics pipeline based on the Galaxy platform, incorporating quality control

and taxonomic assignment tools. Initial quality evaluation of FASTQ files was carried out using FASTA/Q Information Tools and Mothur. Reads were filtered to remove low-quality sequences (length <200 nt), ambiguous base calls, and chimeric sequences. High-quality sequences were grouped into operational taxonomic units (OTUs) at 97% sequence identity, employing a de novo clustering method. Representative sequences from each OTU were classified taxonomically using the Ribosomal Database Project (RDP) Classifier (version 2.5), which assigns taxonomic ranks through a naïve Bayesian algorithm. OTUs identified at 97% similarity were regarded as species-level units, while 95% similarity was applied for genus-level classifications. Furthermore, sequencing reads were concurrently examined using the Illumina BaseSpace 16S Metagenomics App (version 1.0.1), which employs an alternative taxonomic classification pipeline based on the curated May 2013 release of the Greengenes reference database. Only taxes with a relative abundance exceeding 0.1% were included in subsequent statistical analyses.

4.3.7 Statistical analysis

Participants were categorized based on their meditation scores into three levels: low score (0–60; MED 0, 44%), medium score (60–80; MED 1, 28%), and high score (80–120; MED 2, 28%), alongside their Tai Chi and Aikido practice levels. The relative abundances of community members were evaluated using sparse data and summarised at each taxonomic level. The gut microbiome proportions at the phylum and genus levels were assessed using the RDP classifier and the Greengenes database. Bacterial community relative abundance was calculated across phylotypes (% abundance = [number of sequences per phylum or genus / total sequences per sample] × 100). Subsequent analyses disregarded the rarest phyla, specifically those with a relative abundance of less than 0.1% across all samples.

Alpha and beta diversity were computed using PRIMER 7 software at a 97% sequence similarity threshold (Magurran, 2021). For alpha diversity, four complementary indices were calculated using the DIVERSE routine: species richness (S), Margalef's richness index (d), Shannon's entropy index (H′), and Simpson's evenness index ($1 - \lambda'$). Normality of distributions was assessed using the Shapiro-Wilk test. For two-group comparisons (e.g., Aikido vs. Tai Chi, sex), the Mann–Whitney U test was applied, with the rank-biserial correlation coefficient (r) reported as the measure of effect size. For comparisons involving more than two groups (e.g., meditation level, BMI category, physical activity level), the Kruskal–Wallis test was used as a non-parametric alternative to one-way ANOVA, with eta-squared (η^2) reported as the effect size. To control for the inflated risk of Type I error arising

from simultaneous testing, all p-values were adjusted across 20 comparisons using the Benjamini–Hochberg (BH) false discovery rate (FDR) procedure; adjusted p-values (FDR adj p) are reported throughout. All statistical computations were performed using Python (SciPy, Seaborn), and statistical significance was set at FDR adj $p < 0.05$.

For beta diversity, Bray–Curtis dissimilarity matrices were computed and visualised using Principal Coordinate Analysis (PCoA) conducted in PRIMER 7. The statistical significance of group differences in microbiota community composition was examined using two complementary multivariate approaches. First, the non-parametric permutation analysis of similarity (ANOSIM) was applied using PRIMER 7 with default settings. Second, permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001) was performed to formally test the effects of sport type, meditation level, sex, BMI category, physical activity level, and the sport × meditation interaction on microbiota composition. PERMANOVA partitions the total sum of squared Bray–Curtis dissimilarities and evaluates group differences using 9,999 permutations, yielding a pseudo-F statistic and associated p-value; this method is robust to non-normality and provides a direct test of whether group centroids in multivariate space differ significantly. A stepwise approach was used to adjust for potential confounding factors. Data management was performed using IBM SPSS version 25 for Windows (SPSS, Chicago, IL, USA).

4.4 Characterization of Oral Microbiota and Salivary Biomarkers in Tai Chi Practitioners

4.4.1 Study Design and Participants Enrollment

The study protocol was approved by the Institutional Review Board of the University of Rome "Foro Italico" (approval no. CAR 204/2024) and was conducted in accordance with the principles of the Declaration of Helsinki. During recruitment, the aims of the study, experimental procedures, and ethical safeguards including data anonymity, confidentiality, and secure data handling were clearly explained to all prospective participants. Written informed consent was obtained prior to enrolment. Adult Tai Chi practitioners were recruited following their regular training sessions. A total of 19 participants met the inclusion criteria and were selected for the present analysis (Figure 7). Eligibility criteria included regular practice of Tai Chi, willingness to provide salivary biological samples and complete questionnaires, and no use of antibiotics or probiotics, nor any gastrointestinal or oral medical procedures, within the preceding three months. Participants were adults (≥18 years, including individuals >65 years),

and sex distribution. To protect participant privacy, all personal data were anonymized using a unique, non-identifiable alphanumeric code, which was consistently applied to both biological samples and questionnaire records. All inclusion and exclusion criteria including recent antibiotic or probiotic use, chronic diseases, pregnancy, oral inflammatory conditions, and other medical factors potentially influencing oral microbiota or salivary biomarker profiles were assessed during the recruitment phase, prior to enrolment and sample collection.

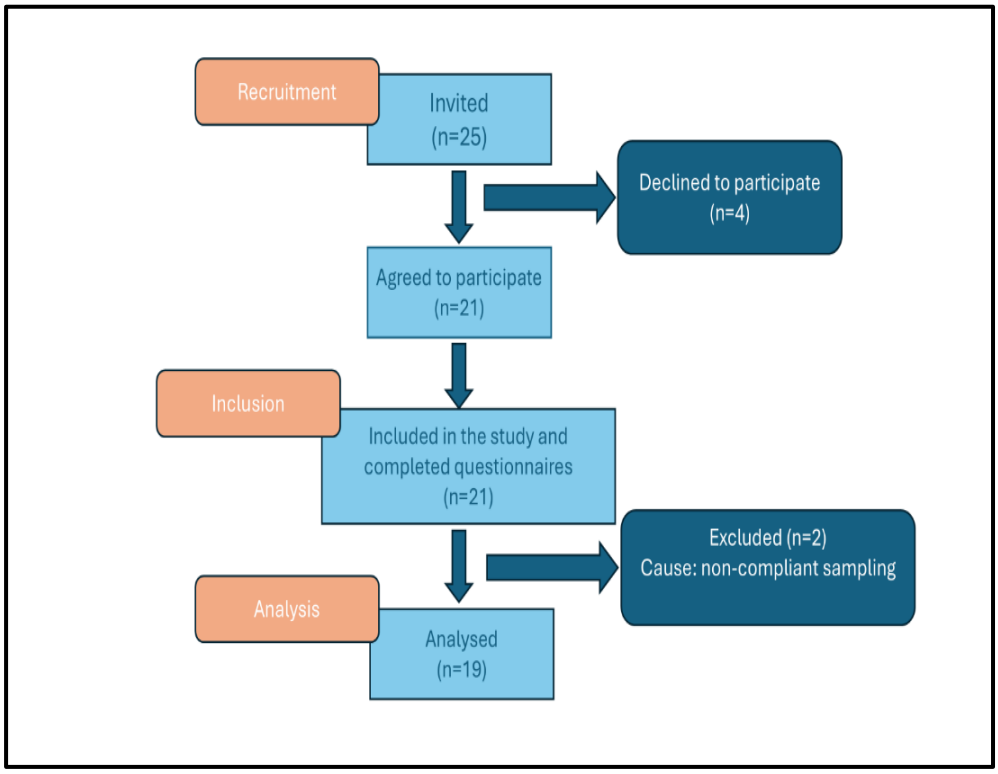


Figure. 7 Flowchart of the study**,** Recruitment, inclusion, and analysis phases of Taichi participants

4.4.2 Questionare and Lifestyle Assesment

Participants completed a structured questionnaire administered via the EU Survey platform, the official European Commission tool for online survey deployment. The questionnaire collected information on age, sex, body weight, height, dietary habits, and lifestyle factors. Provision of this information did not constitute exclusion criteria. Dietary habits and general physical activity levels were assessed using the 9-item questionnaire developed by Martínez-González et al. and the abbreviated version of the International Physical Activity Questionnaire (IPAQ), respectively. Meditation depth was evaluated using the 35-item Meditation Depth Questionnaire (MEDEQ). This instrument assesses both meditation practices (years of experience and session duration) and subjective physical and mental experiences during meditation. Participants rated each item on a 5-point Likert scale ranging from 0 (no agreement)

to 4 (strong agreement). The Meditation Index (MEDI) score was calculated according to the original scoring methodology, integrating factors positively associated with meditation depth and inversely weighted items. The MEDI score reflects qualitative meditation experience rather than time spent meditating. In the present study, all questionnaire-based instruments were employed as exploratory measures to characterize relative differences in meditation depth and general physical activity within the cohort, rather than for diagnostic purposes or population-level comparisons.

4.4.3 Data Collection and Study Design Considerations

Given the exploratory and cross-sectional nature of the study, no formal a priori sample size calculation was performed to power predefined hypothesis-testing comparisons. The final sample size was determined by feasibility and participant availability during the recruitment period. Any power considerations based on paired or longitudinal designs were not applicable and were therefore excluded. Participants received study materials on pre-scheduled days, including an information sheet, informed consent form, written instructions for saliva collection, labelled collection tubes, and access to the online questionnaire. Saliva samples were collected independently by participants following standardized instructions and were kept refrigerated until delivery to the laboratory on designated days and time windows. To ensure anonymization, each participant was assigned a non-identifiable alphanumeric code generated from the first three letters of the mother's name and the father's birth year (e.g., FRA1967). This code was applied to all biological samples and entered the questionnaire, enabling linkage of biological and questionnaire data for integrated analyses while maintaining researcher blinding.

4.4.4 Saliva Sample Collection and Processing

Each participant was provided with standardized instructions for saliva sample collection. In accordance with the cross-sectional study design, a single salivary sample was collected from each participant. Samples were obtained under resting conditions, and participants were instructed to refrain from eating, drinking, smoking, or performing oral hygiene procedures for a defined period prior to collection. Following collection, saliva samples were immediately stored at 4–8 °C in refrigerated containers and transported to the laboratory at the University of Rome "Foro Italico" within 24 h. Upon arrival, samples were processed according to established protocols for downstream microbiota and biomarker analyses. Aliquots were prepared for DNA extraction and ELISA-based biomarker quantification and were kept in the fridge until processing day. Immediately before starting the assay saliva samples were brought

to room temperature and centrifuged at 800 x g for 10 minutes to remove debris. The supernatants were then collected and assayed according to the producer protocol.

4.4.5 DNA Extraction and 16S rRNA Gene Amplification

Salivary specimens were processed according to a validated protocol for extraction of microbial DNA from oral biofluids. Extracted DNA was subsequently purified and normalized prior to sequencing. Amplicon libraries were prepared targeting the V1–V2 region of the bacterial 16S rRNA gene, following the Illumina 16S Metagenomic Sequencing Library Preparation Guide (part# 15044223 Rev. A; Illumina, San Diego, CA, USA). Tagged PCR products were generated using a two-step PCR approach with unique barcoded primer pairs. In the first PCR, locus-specific primers containing adapter overhangs were used to amplify the target 16S rRNA region; the resulting amplicons were then used as template in a second PCR to incorporate sample-specific indices for multiplexing. The primer sequences used for amplification of the V1–V2 region were: 5′-AGAGTTTGATCCTGGCTCAG-3′ (forward) and 5′-TGCTGCCTCCCGTAGGAGT-3′ (reverse). Each PCR reaction was performed in a final volume of 25 µL, comprising 12.5 µL KAPA HiFi HotStart ReadyMix (Roche, Pleasanton, CA, USA), 5 µL of each primer (1 µM), and 2 µL of template DNA. Amplifications were carried out on a Techne® TC-PLUS thermal cycler (VWR International, LLC, Radnor, PA, USA).

4.4.6 Salivary Biomarker Quantification by ELISA

Enzyme-linked immunosorbent assay (ELISA) for S100B (Abcam, ab234573, Cambridge, UK), MMP9 (Abcam, ab246539, Cambridge , UK) and GFAP (Abcam, ab288175, Cambridge, UK) were carried out on the supernatants on duplicates according to the manufacturer's protocol. All the reagents have been prepared fresh the day of the experiment. 50µl of sample or standard were added to appropriate wells: both standards and samples were assayed in double. In linkage phase, 50µl of antibody cocktail were added to all wells and left for incubation at room temperature for 1 hour. In washing process, all wells have been aspirated and added with 350µl of Wash Buffer PT1X three times. In the last phase, 100µl of TMB Development solution were added to each well and, after an incubation of 10 minutes at room temperature in the dark, were added 100µl of Stop solution. Absorbance was measured on a microtiter plate reader (Bio Rad, iMark Microplate Absorbance Reader) at 450nm. The concentration of the three targets in samples was interpolated from the standard curve obtained by the respective standards: the minimal detectable dose (MDD) for S100B is 139pg/ml, for MMP9 is 22.17pg/ml and for GFAP is 68pg/ml.

4.4.7 Illumina Sequencing and Bioinformatics Analysis

Paired-end 16S rRNA gene amplicon sequencing targeting the V1–V2 hypervariable region was performed on salivary samples, and raw FASTQ files were processed on a high-performance computing cluster (Red Hat Enterprise Linux 9). Initial read quality was assessed using FastQC (v0.12.1) and summarized with MultiQC (v1.21). Primer sequences were removed in paired-end mode using cutadapt (v4.7), specifying the forward primer 5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse primer 5′-TGCTGCCTCCCGTAGGAGT-3′, together with their reverse complements (CTGAGCCAGGATCAAACTCT and ACTCCTACGGGAGGCAGCA, respectively). Trimming was performed with 5′-anchored primer matching, a minimum overlap of 10 bp, a maximum error rate of 12%, a minimum post-trimming read length of 50 bp, wildcard matching enabled, and discarding of read pairs in which primers were not detected. Following primer removal, reads were processed using DADA2 (v1.30.0) in R (v4.3.3). Quality filtering was applied using filterAndTrim() with truncation lengths of 120 bp for both forward and reverse reads (truncLen=c(120,120)), maximum expected errors of 2 (forward) and 4 (reverse), removal of reads containing ambiguous bases (maxN=0), quality truncation at Q2, PhiX removal, and multithreading enabled. Error rates were learned separately for forward and reverse reads using learnErrors(), followed by dereplication (derepFastq()) and denoising with the DADA algorithm (dada()). Paired-end read merging was evaluated using mergePairs(), but successful overlap was negligible across samples, consistent with the short post-primer read lengths (~130 bp) relative to the expected V1–V2 amplicon size; therefore, downstream analysis was conducted using a forward-read–only DADA2 workflow. A forward-only amplicon sequence variant (ASV) table was generated using makeSequenceTable() from denoised forward reads, and chimeric sequences were removed using removeBimeraDenovo() with the consensus method. The final non-chimeric dataset comprised 19 samples, 1157 ASVs, and 1,199,493 total reads, corresponding to 98.56% read retention after chimera removal. Taxonomic classification was performed using the SILVA NR99 v138.1 reference database with assignTaxonomy() (reverse-complement matching enabled), and species-level annotation was added using addSpecies(allowMultiple=TRUE); species assignments were interpreted cautiously due to the limited resolution of short 16S amplicons. All outputs were exported in both RDS and CSV formats to enable downstream statistical analyses, including diversity metrics, ordination, and differential abundance testing, which were subsequently performed locally in R.

4.4.8 Statistical analysis

All statistical analyses and visualizations were performed in R using the packages phyloseq, microbiome, tidyverse, ggplot2, rstatix, and ggpubr. A phyloseq object was constructed from the ASV table (1157 ASVs across 19 samples), the corresponding taxonomy table, and sample metadata. Taxonomic profiles were summarized after collapsing features at the genus level and transforming counts to relative abundances (compositional transformation), and stacked bar plots were generated to visualize community composition across grouping variables. Alpha diversity was estimated using phyloseq::estimate_richness(), computing Shannon and Simpson indices, and differences across Sport_level, MEDI_level, BMI, and Gender were explored using boxplots with jittered points and tested using non-parametric methods (Mann–Whitney U test for two-level comparisons and Kruskal–Wallis test for factors with ≥3 levels), given the small sample size and distributional assumptions. Beta diversity was quantified using Bray–Curtis dissimilarity (phyloseq::distance), and ordination was performed by principal coordinates analysis (PCoA) using ordinate(); the percentage of variance explained by the first two axes was calculated from eigenvalues, and PCoA plots were produced with 95% confidence ellipses (stat_ellipse) to illustrate group-level dispersion. Salivary biomarker concentrations (MMP9, S100B, and GFAP) were analysed using non-parametric approaches: MMP9 and S100B were compared across Sport_level and MEDI_level using Kruskal–Wallis tests followed by Dunn's post hoc tests with Benjamini–Hochberg false discovery rate (FDR) adjustment, whereas GFAP frequently below the assay lower limit of quantification (LLOQ = 0.625 ng/mL) was analysed primarily using a detectability framework by classifying samples as detected (≥LLOQ) or below LLOQ and comparing proportions across groups using Fisher's exact test; values below LLOQ were imputed as LLOQ/√2 for visualization only. Where applicable, p-values were adjusted using the Benjamini–Hochberg procedure, all tests were two-sided, and an adjusted p-value < 0.05 was considered statistically significant.

4.5 Metagenome-Assembled Genomes (MAGs) analysis

4.5.1 Metagenomic sample collection

To conduct this study, a preliminary screening of publicly available studies was conducted to identify shotgun metagenomic datasets suitable for genome-resolved analysis of the gut microbiome in physically active and sedentary populations. Studies were considered eligible if they included human faecal shotgun metagenomic data, involved athletes or physically active individuals from clearly defined sport disciplines, provided accessible raw sequencing data through public repositories such as NCBI/SRA or ENA, and contained sufficient metadata to

allow classification according to sport type or exercise-intensity category. Studies based only on 16S rRNA sequencing, studies involving clinical or diseased populations, datasets without accessible raw reads, and studies lacking relevant exercise or lifestyle metadata were excluded. After screening the available literature and public repositories, six datasets were selected for inclusion because they represented distinct sport modalities and allowed comparison with a sedentary control group. So we collected six publicly available metagenomic datasets including a variety of different sports. These datasets include data from runners (PRJEB46398) (CM et al., 2025), rugby players (PRJEB15388) (Barton et al., 2018), cricketers (PRJEB28338) (CM et al., 2020), cyclist (PRJNA305507) (Petersen et al., 2017), rowers and runners (PRJNA472785) (Scheiman et al., 2019) and the sedentary control group (PRJEB20054) (Cronin et al., 2018). These six publicly available shotgun metagenomic cohorts representing distinct sport modalities were integrated, including runners, rugby players, cricketers, cyclists, rowers/runners, and sedentary controls and samples were grouped using an ACSM-based classification scheme (shown in Figure 8) to enable cross-cohort comparisons of microbiome composition and genome-resolved features across exercise-intensity categories.

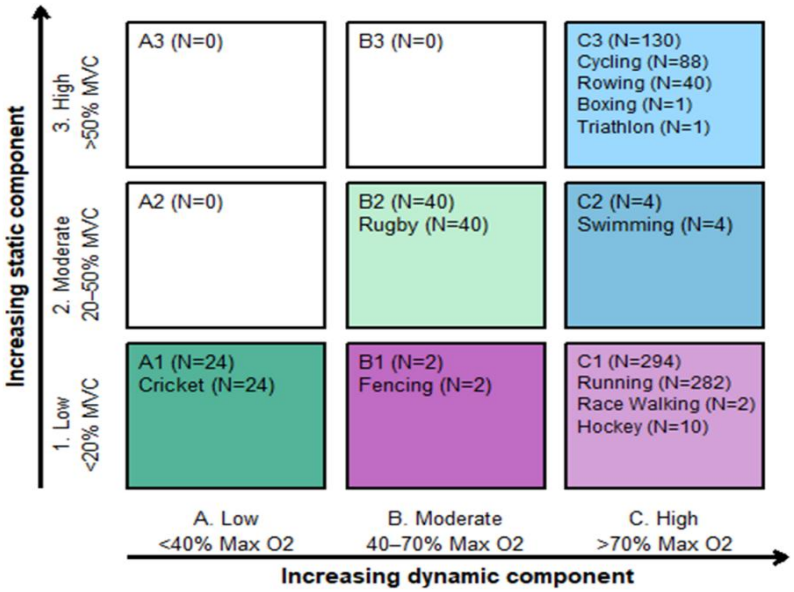


Figure. 8 Exercise-Intensity Classification Using ACSM Athlete Categories

Based on this classification we divided the data into total five groups including the A1, B2, C1, C3 and S as sedentary control.

4.5.2 Metagenomic Data Processing & Taxonomic Profiling

The processing of metagenomic data performed by using the Teagasc high-performance computing cluster. The raw reads were collected from the NCBI. These raw reads then processed to proceed with the analysis.

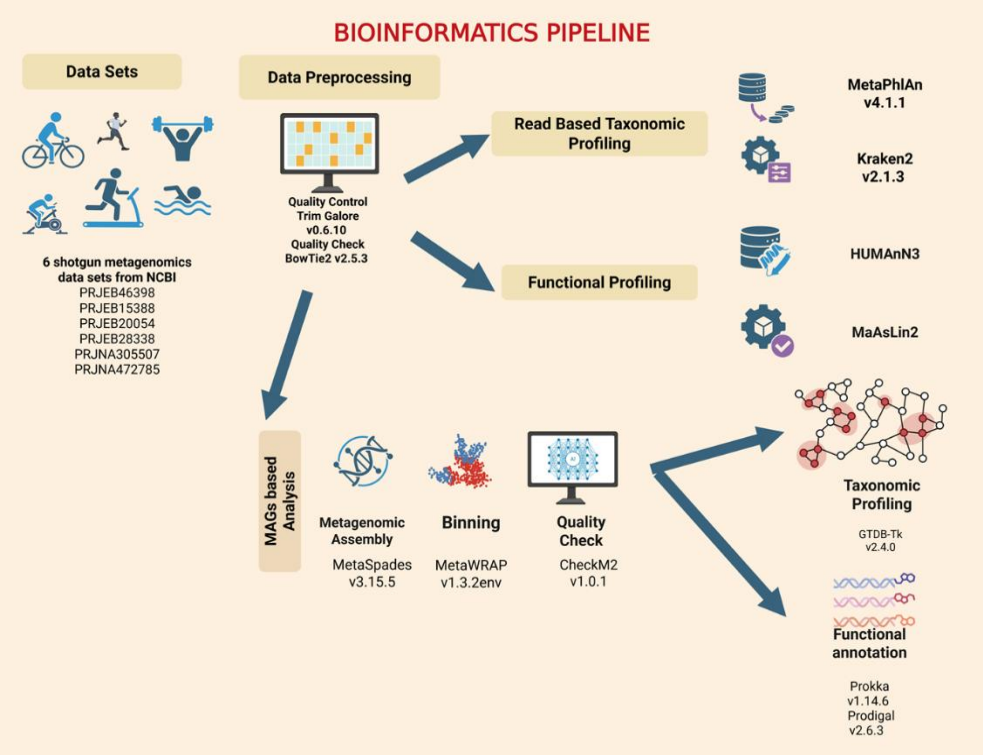


Figure. 9 Integrated Metagenomic Workflow for Athlete Microbiome Profiling

It includes various steps, (shown in Figure 9) and the first step is quality control of raw reads. Quality control includes different step, and the first step is performed by using the Trim Galore v0.6.10 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and this step discards the low-quality reads with a quality score less than 20, base pairs less than 75 and the reads with more than 2 excessive ambiguous nucleotides. The second step for quality check was removing host contamination like Human (HG19) and bacteriophage phiX174 DNA, and it was performed by mapping the reads to the respective reference genomes by using the BowTie2 v2.5.3 (Langmead & Salzberg, 2012; Langmead et al., 2009) and at the last reads are sorted into forward and reverse files and used for downstream analysis for performing taxonomic profiling and metagenome assembled genome analysis. The taxonomic profiling was performed using the MetaPhlAn v4.1.1, utilizing the MetaPhlAn 4 database to generate taxonomic read-mapping-based profiles and further Kraken2 v2.1.3 also used for taxonomic profiling by utilizing a k-mer-based approach for rapid and accurate species identification.

The bioinformatics tools described above were implemented using scripts sourced from the

GitHub repository: https://github.com/SegataLab/MASTER-WP5-pipelines. These scripts were adapted and customized from the repository's collection to suit the specific requirements of this study.

4.5.3 Extraction of MAGs and Taxonomic Assignment

A validated pipeline used for generating the metagenomic assemblies (Pasolli et al., 2019) which included the following steps: (i) metagenomic assembly using MetaSpades v3.15.5; (ii) removal of contigs shorter than 1,000 bp; (iii) alignment of remaining contigs to the original raw data using Bowtie2 v2.5.3 to calculate coverage information; (iv) binning of contigs using MetaWRAP v1.3.2env; (v) quality control (QC) of the resulting putative genomes using CheckM2 v1.0.1 (Chklovski et al., 2022), with subsequent filtering to retain high-quality (HQ) (completeness > 90% and contamination < 5%) and medium-quality (MQ) (completeness ≥ 50% and contamination < 5%) MAGs (Bowers et al., 2017). Only HQ MAGs were carried forward for further analysis. MAGs derived from the control metagenomes, representing participants with a sedentary lifestyle, were pooled into a single group for comparison. Functional annotation of the HQ MAGs was performed using Prokka v1.14.6 and Prodigal v2.6.3 (Seemann, 2014). The taxonomic classification of recovered HQ MAGs and the identification of potentially novel species were achieved using GTDB-Tk v2.4.0 (Chaumeil et al., 2022), utilizing the MASH sketch database. High quality MAGs annotated using Prokka and Prodigal.

4.5.4 Statistical Analysis & Data Visualization

Statistical analyses were conducted in R v4.1.2 using RStudio. For diversity analyses, the following approaches were used: Alpha and Beta Diversity: Community-level diversity metrics (Shannon and Simpson indices) were computed using the microbiome package. Differences between groups were assessed using the Kruskal-Wallis test (for alpha diversity) and PERMANOVA (Adonis) using the vegan package for beta diversity. Pairwise Comparisons: Pairwise comparisons of the groups were conducted using the pairwiseAdonis v0.4 package, with Benjamini-Hochberg correction for multiple comparisons. Multivariate Analysis: Clustering and ordination were visualized using NMDS (Non-metric Multidimensional Scaling), with the relationship between microbiome composition and metadata variables (e.g., sport, gender) analyzed using envfit in the vegan package. Gene Association: Gene presence and absence were evaluated using Roary (for gene clustering) and Scoary (for trait association analysis). Associations with exercise status were computed using pairwise comparison algorithms, with Bonferroni-adjusted P values < 0.05 considered significant. Data

Visualization: Data visualization, including diversity plots and association heatmaps, was conducted using ggplot2.

5 RESULTS

5.1 RESULTS OF LITERATURE REVIEW

5.1.1 Systematic review and metanalysis: The role of gut microbiota in different types of physical activity and their intensity

Three databases (Pubmed, Scopus and Science direct) were investigated. In total, 1318 studies were retrieved from all searched databases and, after expelling duplicates, 127 articles remain for the following steps. Of the remaining studies, 100 were selected for full-text analysis. Then, the full texts of selected articles were accessed and evaluated considering the inclusion and exclusion criteria. After the assessment, 81 articles were excluded based on the exclusion criteria. Finally, 19 articles fulfilled the inclusion criteria and were placed in the systematic review, and 10 articles were used for meta-analysis as shown in figure 10.

Based on the evidence summarized in previously published narrative reviews, a meta-analysis was performed, focusing on a limited number of studies that met the inclusion criteria for the systematic review. The studies included in the systematic review were categorized into two groups: one comprising animal studies (Table 1), and the other comprising human studies (Table 2). The animal studies included in the systematic review were published between 2017 and 2021, indicating a stable research output in this area during that period. These studies reflect a global scope, having been conducted in multiple countries, including Finland, the USA, Taiwan, China, and France. Across all four animal studies, a cohort study design was used to assess outcomes. Sample sizes ranged from 20 to 100 animals, and the ages of the subjects spanned from 5 weeks to 12 years, enabling comparisons between younger and more mature groups. Overall, the study populations were predominantly male (80%), with females comprising 20%.

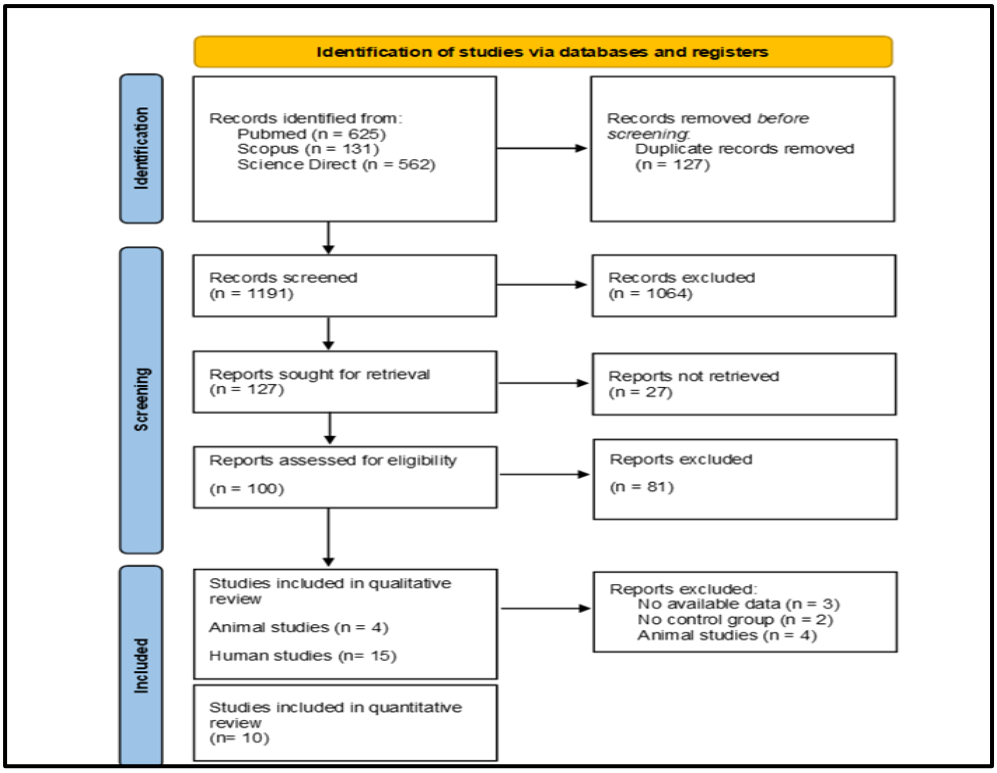


Figure. 10 PRISMA flow diagram following the PRISMA guidelines (Ghaffar et al., 2024)

The exercise interventions evaluated in the animal studies included exhaustive swimming (with a weight attached to the tail to induce fatigue), combined endurance and treadmill training to assess additive effects, endurance exercise alone, and treadmill exercise alone. Collectively, these studies assessed how different exercise modalities influence gut microbiota composition and related outcomes.

For the human studies, all included articles were also published between 2017 and 2021 and were conducted across a wide range of countries, including China, the USA, Ireland, Poland, France, Portugal, Australia, New Zealand, Canada, Germany, and Korea. Overall, the literature suggests a marked increase in research interest in the relationship between physical activity and the gut microbiota, reflecting growing attention to its health relevance. The included human studies employed diverse designs, including randomized controlled trials, cross-sectional studies, cohort studies, and clinical trials. Sample sizes ranged from 2 to 90 participants, and participant ages spanned from 12 to 72 years, allowing assessment across a broad age spectrum. Across these studies, approximately 60% of participants were male and 40% were female. Quality assessment was performed using design-appropriate tools. Cross-sectional studies were evaluated using an adapted Newcastle–Ottawa Scale for cross-sectional studies, with 83% rated as fair quality and indicating a moderate risk of bias. Cohort studies were assessed using the

Newcastle–Ottawa Scale (NOS) for cohort studies, showing low risk of bias in 45% of articles and moderate risk in 55%. Randomized clinical trials were evaluated using the Cochrane Collaboration Risk of Bias tool for randomized trials (RoB 2).

Table 1. Characteristics of original studies and main results related to physical activity and microbiota in animals.

Author, Year, Ref	Study design	Subjects (animals)	Sample size	Age (Mean value)	Gender	Type of exercise	Duration, frequency, training volume	Outcome
(Pekkala et al., 2017)	Cohort study	Mice	20	7 weeks	Male and female	Endurance exercise treadmill running	ND	Intrinsic aerobic capacity governs microbiomes, which may influence body weight, metabolism, and gene expression.
(Yang et al., 2021)	Cohort study	Mice	30	5 weeks old	Male	Moderate intensity treadmill exercise	3 days a week, 10–20 minutes a day, slope 0, speed 10–13 mile per minute	Moderate-intensity treadmill exercise significantly increased the exercise capacity of mice and alters the core bacteria and bacterial metabolic activities
(Mach et al., 2021)	Cohort study	Horse	20	10±1.6 9 years	Male and female	Endurance exercises	120 and 160 km race	Targeting the gut-mitochondria axis, therefore, appears to be a possible strategy to enhance athletic performance
(Tung et al., 2019)	Cohort study	Mice	100	7 weeks old	Male	Exhaustive swimming	exhaustive swimming test, with 5% body weight (BW) loading on the tail	The mice with various intrinsic exercise capacities have different gut microbiome as well as transcriptome and proteome of soleus muscle by applying multi-omics approaches. The main bacteria and controlling factors, including miRNA and functional proteins, may be too much correlated with the adoption of physiological functions and exercise capacity.

Table 2. Characteristics of original studies and main results related to physical activity and microbiota in humans.

Author, Year, Country	Study design	Sample size	Age (Mean value ± SD)	Gender	Type of exercise	Duration, frequency, training volume	Outcome
(Petersen et al., 2017)	Cross-sectional study	33	19–49	Male and female	Cycling	All participants spent a minimum of 6 h exercising per week	No significant correlations were found between taxonomic cluster and being either a professional or amateur level cyclist, high abundance of the genus *Prevotella* (≥2.5%) was substantially correlated with time noted for exercising during an average week.
(Karl et al., 2017)	RCT	73	>18	Male and female	Fifty-one kilometers of cross-country ski-march	4 days arctic military training (51-km cross-country ski-march, during which volunteers skied in the 50:10-min work-to-rest ratios also carrying a ~45-kg pack).	Physiological stress is associated with the intestinal permeability, changes in gut microbiota by modifying diet and stress level can change intestinal permeability
(Yang et al., 2017)	Cross-sectional study	71	19-49	Female	ND	2-min warm-up at 50 W to access VO2 max	Cardiorespiratory fitness is associated with gut microbiota composition, independent of age and carbohydrate or fat intake.
(Tabone et al., 2021)	Cohort study	40	18-50	Male	Cross-country running	10-min warm-up of continuous running on a treadmill at 60% of their maximum heart rate. After the warm-up, they ran with a slope of 1% at a speed of 10 km/h, with increase of 0.3 km per hour every 30 second until volitional exhaustion.	The performance of a single exercise bout in cross-country non-professional athletes produces significant changes in the microbiota, in serum and fecal metabolome, which may have health implications
(Oliveira et al., 2022)	Cohort study	17	18-25	Female	Football	Perceived exertion method	The physical and physiological requirements of training and matches of an official international tournament did not vary the gut microbiota balance of elite female football players.
(Craven, Cox, Bellinger,	Cohort study	14	18-25	Male and female	Middle distance running	Three weeks of normal training; 3 weeks of high-	The alpha-diversity and global formation of the gut microbiome were

et al., 2022)						volume training (10, 20 and 30% increment in training volume during every successive week from NormTr); one-week taper (TaperTr; 55% exponential decrease in training volume from third HVolTr week).	unaffected by differences in training volume. However, an increment in training volume led to various changes at the lower taxonomy levels that did not come back to pre-HVolTr levels following the taper period
(Moitinho-Silva et al., 2021)	RCT	42	20 – 45	Male and female	Strength exercise, endurance exercise	The participants warmed up for five minutes on the treadmill, ergometer, or rowing machine before beginning their 30-minute training session. One session included six distinct exercises, two for each leg, chest, and back, to develop the big and main muscle groups.	Various types of exercise have different but balanced effects on the overall physiology of humans and very versatile microbial changes in the gut
(Han et al., 2020)	Cohort study	19	12-26	Female	Rowing	ND	Gut microbial communities depend upon the type of exercise associated with dietary factors and physical characteristics.
(Liang, Zhang, & Peng, 2019)	Cross-sectional study	31	20-24	Male and female	Professional martial arts	Higher-level group: Exercise load (hours/week):29.25± 9.48; Lower-level group: 16.63±6.82	The higher-level athletes had increased diversity and higher metabolic proportions of the gut microbiome for it may positively impact athletic performance.
(Cronin et al., 2018)	RCT	90	18-40	Male and female	Aerobic and resistance exercise training	Eight-week combined aerobic and resistance exercise training program	The improved body composition with exercise does not depend on basic changes in the diversity of microbial populations in the gut. The various microbial characteristics already observed in long-term habitual athletes may be a late response to exercise and fitness enhancement.
(Kulecka et al., 2020)	Cross-sectional study	71	14-72	Male and female	Marathon running; cross-country skiing	Highest level of training endurance sports athletes trained on average 1.58 ± 0.58 times per day, taking 7.25 ± 2.17 training units	Excessive training is involved with the changes in composition and elevation of increased bacterial diversity.

						in one week lasting 2.79 ± 1.74 hour per day on an average	
(Rettedal et al., 2020)	Cross sectional study	29	20-45	Male	HIIT training	Three weeks of high intensity training (8–12 × 60 s cycle ergometer bouts at VO2 maximum power output interspersed by 75 second rest, three times per week)	The gut microbiome's overall composition is not significantly altered by short-term HIIT; however some microbiome genera are linked to insulin sensitivity measures, and HIIT enhanced these markers in overweight subjects.
(Estaki et al., 2016)	Cross sectional study	39	18-35	Male and female	Not specific	ND	Cardiorespiratory fitness is correlated with increased microbial diversity in healthy humans and that the associated changes are anchored around a set of functional cores rather than specific taxa.
(Jang et al., 2019)	cross-sectional study	45	25±3 (bodybuilders); 20 ±1 (distance runners) Healthy men 26 (±2) years	Male	Body building; distance running	ND	Athlete type was significantly associated with the relative abundance of gut microbiota at the genus and species level: *Faecalibacterium*, *Sutterella*, *Clostridium*, *Haemophilus*, and *Eisenbergiella* were increased ($p < 0.05$) in bodybuilders, while *Bifidobacterium* and *Parasutterella* were decreased ($p < 0.05$).
(Barton et al., 2021)	Cohort study	2	31.5	Male	Marathon and triathlon	Regular aerobic exercise complimented with twice weekly resistance training	Sustained fitness improvements support alterations to gut microbiota and physiologically relevant metabolites.

Different types of physical activity can influence the gut microbiota in distinct ways. Accordingly, the studies included in the systematic review encompassed a wide range of athletes and sports, including running, cycling, strength and endurance training, bodybuilding, rowing, skiing, football, aerobic and resistance training, martial arts, long-distance cross-country ski events (51 km), and high-intensity interval training (HIIT). Collectively, these studies provided a broad framework for evaluating gut microbiota diversity across multiple exercise modalities and training intensities.

Table 3 summarizes the studies included in the meta-analysis, describing microbiota profiles

and the alterations associated with physical training. It also categorizes physical activity intensity for both control and experimental groups across all included studies. Overall, 10 human studies were included. In most studies (80%), fecal samples were collected following chronic adaptation to regular training over time, whereas in the remaining studies (20%) sampling was performed shortly after training (immediately up to one-week post-exercise).

Across the included studies, fecal microbiota profiling was primarily performed using 16S rRNA gene amplicon sequencing on the Illumina MiSeq platform, with one study using an Ion Torrent PGM sequencer. All studies targeted the V3–V4 region, although primer sets differed between studies, including both sequencing and universal primers. All ten studies assessed both alpha and beta diversity to characterize microbial biodiversity, and an increase in the Shannon index was generally observed in groups performing higher-intensity physical activity. In contrast, diversity appeared largely unchanged across different aerobic exercise types or intensity levels in some studies. Several studies also reported an increase in the Bacteroidetes-to-Firmicutes ratio (B/F ratio) among physically active participants. Specifically, 50% of the studies (5 out of 10) observed an increase in this ratio, and in four of these cases it was associated either with higher training intensity (from low/moderate to vigorous) or with a greater overall exercise volume (e.g., increased hours of training per week).

Table 3: Characteristics of samples and specificities included studies in metanalysis

Author, Year	Biodiversity indicators, Alpha diversity	Biodiversity indicators, Beta diversity	B/B ratio	Firmicutes	Bacteroidetes	Diversity	Comparison between groups	Firmicutes/ Bacteroidetes %	Type of exercise and control	Classification of Exercise and Sport Intensity based VO_2max and details		Classification of Exercise and Sport Intensity based METs values and details	
										CON	PA	CON	PA
(Craven, Cox, Bellinger, et al., 2022)	↔Alpha-diversity (Shannon Index, Chao1)	Microbial communities are similar	A) CON= 1.3; PA=0.7; B) CON=1.2 ; PA=0.8	High-volume training: ↓ Lachnoclostridium (p = 0,02), S. parasagunis (p =0,02) ↑ R. callidus (p = 0,03)Lachnoclostridium (Firmicutes) 21% H. parainfluenzae (pseudomonadota) 0.5% S. parasanguinis (Firmicutes) 3% R. callidus (Firmicutes) 15%	ND	Only three types of Firmicutes identified including Lachnoclostridium, S. parasanguinis and R. callidus. No Bacteroidetes present.	Faecal samples were obtained before and instantly after each training (three weeks of the normal training, three weeks of the high-volume training and a one-week taper training)	Pasteurellaceae (pseudomonadota) 0.5% Haemophilus (pseudomonadota) 0.5% Lachnoclostridium (Firmicutes) 21% H. parainfluenzae (pseudomonadota) 0.5% S. parasanguinis (Firmicutes) 3% R. callidus (Firmicutes) 15%	Middle distance running CON=Normal training; PA= Higher level training	A) Vigorous (60-84 % VO_2R) B) Vigorous (60-84 % VO_2R) + Normal Training volume	A) Vigorous (60-84 % VO_2R) + Training volume increasing during the activity	A) Vigorous > 6 METs; B) Vigorous > 6 METs	A) Vigorous >6 METs; B) Vigorous >6 METs
(Cronin et al., 2018)	α-diversity Shannon index changes were non-significant (no α-diversity value provided)	Microbial communities are statistically different. Bray-Curtis's dissimilarity β-diversity changes were non-significant	CON=0.02; PA=0.0015	Borrelia hermsii (Spirochaetota)2.3% Mycoplasma pneumoniae (Tenericutes) 6.6%; Streptococcus thermophilus 0.002%	ND	In training group the Firmicutes are Streptococcus thermophillus and Lactobacillus lactis. and in one training group Firmicutes are only Streptococcus thermophillus	Fecal samples collected before and after 8 weeks intervention (exercise only group, exercise and protein supplement group and control	Only exercise group: Borrelia hermsii (Spirochaetota) 2.3%; Mycoplasma pneumoniae (Tenericutes) 6.6%; only protein group: Streptococcus thermophilus	Aerobic and resistance exercise training CON=Sedentary; PA=exercise	Low (20-39 % VO_2R)	High (60-84 % VO_2R)	Vigorous > 6 METs (METs consumption related to daily life)	Vigorous >6 METs

						group)	0.002%					
(Estaki et al., 2016)	↑*Shannon, Chao1, Simpson ↑Alpha diversity (Chao, Shannon, Simpson)	Microbial communities are statistically different. Beta diversity (Bray–Curtis)	CON=1.4; PA=0.53	↑* Lachnospiraceae ↑* Christensenellaceae, ↑* Ruminococcaceae↑*Clostridiales.(p=0.026), Roseburia , Lachnospiraceae, Erysipelotrichaceae	ND		Fecal samples were collected in healthy adults before and after one week of VO2 testing	Erysipelotrichaceae, Coprococcus, Roseburia, Adlercreutzia, and unknown members of Clostridiales, Lachnospiraceae, and Erysipelotrichaceae.	CON=lower level exercise; PA=higher level exercise	Low (20-39 % VO_2R)	High (60-84 % VO_2R)	Vigorous > 6 METs (METs consumption related to daily life)	Vigorous >6 METs
(Han et al., 2020)	↑*Shannon, Simpson ↑Alpha diversity (Shannon, Simpson)	Microbial communities are statistically different. Beta diversity (Jac card, Unifrac), ↑B/B	CON=2.31; PA=3.89	↑*Ruminococcaceae, Clostridiales, Faecalibacterium and Lachnospiraceae.	↑*	Firmicutes included are Ruminococcaceae, Clostridiales, Faecalibacterium and Lachnospiraceae.	Fecal samples were collected from adult elite athlete's (AE), youth elite athlete's (YE), and youth non-elite athlete's (YN) during two months period.	The average relative abundances of Firmicutes and Proteobacteria of AE (76.27% and 8.73%) and YE (64.7% and 10.69%) were higher than those of YN (58.12% and 8.01%), the moderate relative richness of Bacteroidetes	Rowing CON=non elite athletes; PA= elite athletes	Moderate (40-59 % VO_2R)	Vigorous (60-84 % VO_2R)	Vigorous > 6 METs	Vigorous >6 METs
(Jang et al., 2019)	↓*Shannon, OTUs, Chao1 Diversity between groups: → Alpha diversity (Chao1) ≠ Beta diversity (PC) Alpha and beta	Microbial communities are statistically different	A) CON=4.79; PA=15.69; B) CON=4.79; PA=3.96	Faecalibacterium, Sutterella, Clostridium, Haemophilus, and Eisenbergiella were increased in ($p < 0.05$) in bodybuilders, while Bifidobacterium and Parasutterella were the decreased ($p < 0.05$)	ND	The Firmicutes compromised of Blautia wexlerae, callidus,Faecalibacterium_uc Faecalibacterium prausnitzii, Clostridium	Fecal samples were obtained from sedentary men as control and from body builders and	Faecalibacterium, Sutterella, Clostridium, Haemophilus, and Eisenbergiella were the highest ($p < 0.05$) in bodybuilders,	Body building; distance running A) CON=not exercise; PA=body building; B) CON=not exercise;	A) Low (20-39 % VO_2R) B) Vigorous (60-84 % VO_2R)	A) Vigorous (60-84 % VO_2R) B) Vigorous (60-84 % | A) light < 3 METs B) Vigorous > 6 METs	A) Vigorous >6 METs B) Vigorous >6 METs

	diversity remains same in both groups					innocuum Eubacterium hallii, Ruminococcus ,, Weissella confusa, Lactobacillus sakei.	distance runners	while Bifidobacterium and Parasutterella were the lowest ($p < 0.05$	PA=distance running		VO_2R)		
(Karl et al., 2017)	↑*Shannon, Chao1 ↑Alpha diversity (Shannon) → Alpha-diversity (Chao1, OTU) ↑B/B ratio	Bray-Curtis distances	CON=1.28; PA=1.86	Pepto-streptococcus, Christensenella, Faecalibacterium, Staphylococcus, unassigned taxa within the Mogiobacteriaceae, Christensenellaceae, and Planococcaceae families	↓*	↑*	Fecal samples were collected from subjects the control group, the protein supplemented group and carbohydrate supplemented group two days before and one day after the stress exercises	Pepto-streptococcus, Christensenella, Faecalibacterium, Staphylococcus, unassigned taxa within the Mogiobacteriaceae, Christensenellaceae, and Planococcaceae families. Verrucomicrobia*	Fifty one kilometer of cross-country ski-march CON=Pre-Training; PA=Post-Training	Vigorous (60-84 % VO_2R) [61]	Vigorous (60-84 % VO_2R) [61]	Vigorous > 6 METs	Vigorous >6 METs
(Kulecka et al., 2020)	Chao1, Simpson (p=0.025 & p=0.00059) ↑Alpha diversity (Shannon, Simpson, Chao1), ↑B/B ratio	Microbial communities are statistically similar	CON=1.83; PA=10.04	Bacteroides, Prevotella, Alistipes, Sutterella and Subdoligranulum, Ruminococcaceae family and Barnesiella genus, Firmicutes; Clostridia; Clostridiales; Lachnospiraceae; Lachnoclostridium	Bacteroidetes↑* Prevotella		Stools samples were obtained from the 14 marathon runners, 11 cross country skiers and the 46 inactive healthy controls.	Bacteroides, Prevotella, Alistipes, Sutterella and Subdoligranulum, Ruminococcaceae family and Barnesiella genus, Firmicutes; Clostridia; Clostridiales; Lachnospiracea e	Marathon running; cross-country skiing CON=lower level exercise; PA=higher level exercise	Low (20-39 % VO_2R)	Vigorous (60-84 % VO_2R)	A) light < 3 METs	Vigorous >6 METs

(Liang, Zhang, & Peng, 2019)	↑*Shannon (p=0.019), Simpson(p=0.001)	Microbial communities are statistically different	CON=1.9; PA=0.568	↑*Parabacteroides (p<0.001) Oscillibacter (p=0.026 Bilophila (p=0.036) Megasphaera (p=0.04) Phascolarctobacterium (p=0.028)	↑*Megasphaera(p=0.04)	Veillonellaceae, Phascolarctobacterium, Oscillibacter and Megasphaera. The Bacteroidetes are Porphyromonadaceae and Parabacteroides.	Fecal samples were collected from 12 large scale and 16 small scale athletes	Higher level group: Porphyromonadaceae 4.4%; Veillonellaceae 0.9%; Parabacteroides 2.3%; Phascolarctobacterium 2.2%; Oscillibacter 0.7%; Megasphaera 0.008%; Bilophila 0.3%; Lower-level group: Porphyromonadaceae 2%; Veillonellaceae 4.5%; Parabacteroides 0.8%; Phascolarctobacterium 0.5%; Oscillibacter 0.3%; Megasphaera 0.069%; Bilophila 0.075%	*Professional martial arts CON=Lower level group; PA=Higher level group*	Moderate (40-59% VO_2R) + Lower-level of Exercise load (hours/week)	Moderate (40-59% VO_2R) + Higher level of Exercise load (hours/week)	Vigorous > 6 METs	Vigorous >6 METs

(Oliveira et al., 2022)	↑*Shannon, Chao1, Simpson (P=0.013)	Microbial communities are statistically different	CON=1.78; PA=1.677	↑*Collinsella aerofaciens↑*Faecalibacterium prausnitzii	↑*Prevotella Copri (31%)	Firmicutes included are Fecalibacterium and Bacteroidetes included is Prevotella	Fecal samples were collected from subjects on 2nd, 3rd, 9th and 10th day of matches	At the beginning, 50% of bacteria were Firmicutes (Frimicutes) followed by Bacteroidetes (28%) and Actinobacteria (19%). Firmicutes (Frimicutes) (52%) was the most prevalent type overall at the conclusion of the event followed by Bacteroidetes (31%) and Actinobacteria	Football CON=Baseline; PA=End of study	Moderate (40-59% VO_2R)	Vigorous (60-84 % VO_2R)	Vigorous > 6 METs	Vigorous >6 METs
(Petersen et al., 2017)	↑* Shannon (p = 0.0004), OTUs	Microbial communities are statistically different	CON=2.05; PA=2.33	NA	↑* Prevotella	NA	Fecal samples were obtained from 33 cyclists with extensive medical issues and no antibiotic use within the last year.	NA	Cycling PA=professional level (the highest level) and CON=amateur level	Vigorous (60-84 % VO_2R) + lower level of Exercise load (h/week)	Vigorous (60-84 % VO_2R) + higher level of Exercise load (h/week)	Vigorous > 6 METs	Vigorous >6 METs

In 30% of the studies included in the meta-analysis, comparisons were made between the same exercise intensities, while in 60% of the studies, comparisons were conducted between different intensity levels. Specifically, 40% compared low intensity (below 30% of VO2 max) with vigorous intensity (above 60% of VO2 max), and 20% compared moderate intensity (between 30% and 60% of VO2 max) with vigorous intensity (above 60% of VO2 max). In the piechart shown in the figure 11 the study designs and the varying intensity levels investigated were categorized into three exercise intensity levels: low, moderate, and vigorous. These levels were used to classify the comparisons made between different intensities, helping to examine the effects of exercise at each intensity.

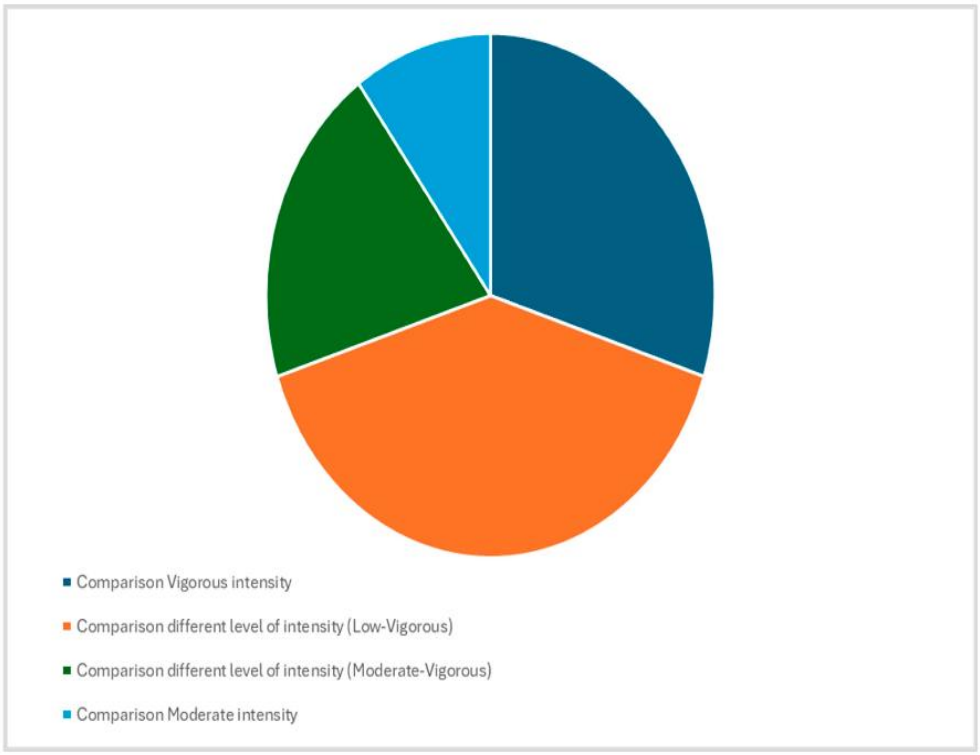


Figure 11 Pie chart illustrates the design of studies and the different levels of intensity investigated categorized by exercise intensity levels (Ghaffar et al.,2024)

The meta-analysis used the standardized mean difference (SMD) as the summary effect measure. Ten studies were included, and two of them contributed results for two separate populations, which were treated as independent estimates. Individual SMDs ranged from −0.7583 to 3.5830, with 83% of estimates indicating a positive effect. Using a random-effects model, the pooled effect was $\hat{\mu} = 0.7597$(95% CI: 0.1764–1.3429), which was significantly different from zero (z = 2.5527, p = 0.0107). Heterogeneity was substantial (Q (11) = 170.5522,

$p < 0.0001$; $\tau^2 = 0.9827$; $I^2 = 94.4979\%$). The 95% prediction interval for the true effects was −1.2689 to 2.7882, suggesting that although the overall mean effect was positive, some studies may plausibly show a negative true effect as shown in figure 12.

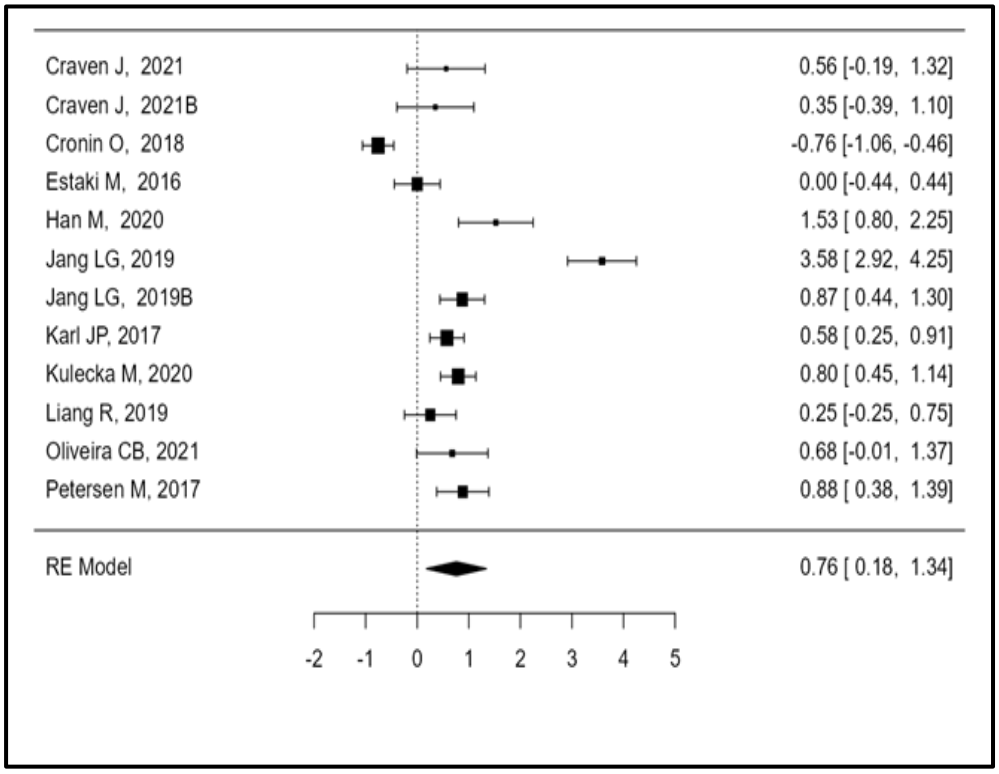


Figure 12. Metanalysis Forest plot: the differences in the means of the Firmicutes/Bacteroidetes (Ghaffar et al.,2024)

The Firmicutes/Bacteroidetes ratio (formerly Firmicutes/Bacteroidetes), which is often used as a broad indicator of gut microbial balance, was evaluated. This ratio has been proposed as a practical biomarker of dysbiosis because multiple studies have reported differences between normal-weight and obese individuals. As a result, the B/B ratio is frequently discussed in the literature as a proxy for obesity or suboptimal health status, although this interpretation remains debated within the scientific community. In the present study, as shown in Table 3 and Figure 13, the B/B ratio varied according to exercise intensity, indicating that different training loads can influence gut microbiota ratios moreover a funnel plot indicating the distribution of studies for publication bias.

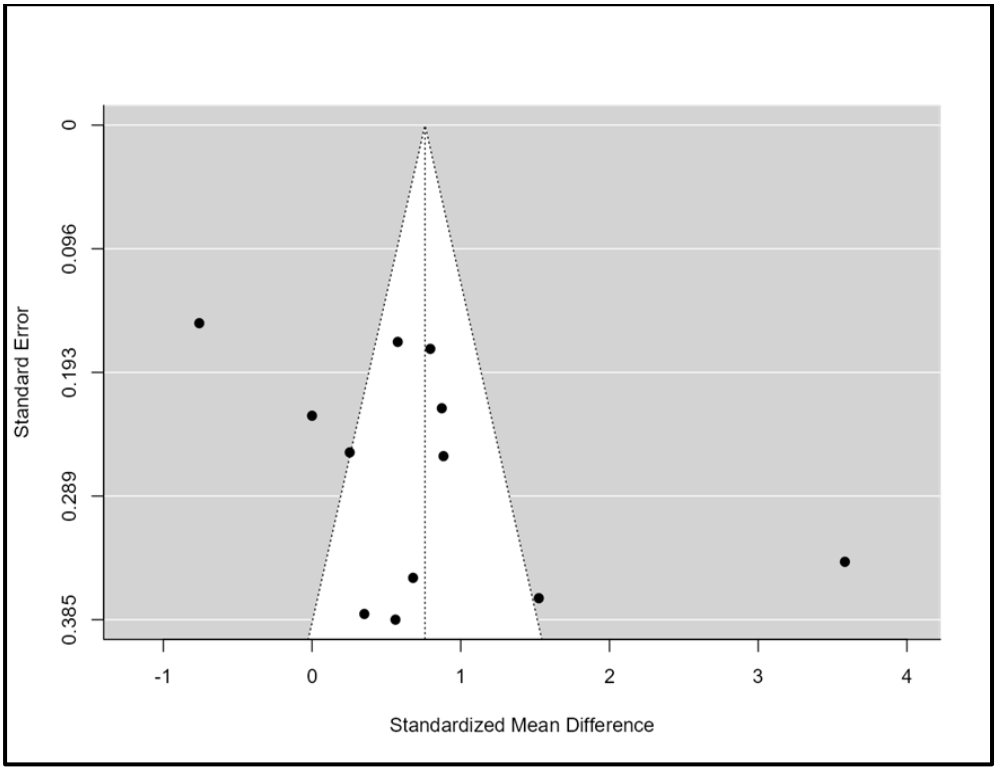


Figure 13. The funnel plot showing the distribution of studies for publication bias (Ghaffar et al.,2024)

An assessment of the residuals indicated that one study had a residual exceeding ±2.8653, suggesting that it may represent a potential outlier and could be unduly influential within this model. No evidence of funnel plot asymmetry was detected using either the regression test or the rank-correlation test ($p = 0.1256$ and $p = 0.6384$, respectively; Figure 13). To evaluate the influence of potential moderators on the *Firmicutes/Bacteroidetes* (B/B) ratio, subgroup analyses and meta-regressions were conducted. The meta-regression examined intervention characteristics (aerobic vs anaerobic; intensity and duration), sample size, publication year, methodological quality, and WHO region of origin. The results indicated that intervention type significantly affected the *Firmicutes/Bacteroidetes* ratio in adults ($p = 0.004$), and that exercise intensity was a key determinant of these ratios. Duration showed a similar pattern only when associated with vigorous-intensity exercise, although this effect was not statistically significant ($p = 0.410$).

The literature review shows that different physical activity patterns can have different effects on the gut microbiota and, in turn, on athletic performance. The interaction of different physical activities like strength exercises and endurance exercises and found that typologies of exercise have different but moderate impacts on the overall human physiology and very distinct microbial modifications in the gut; the physically active group shows significantly more of the

genera *Coprococcus*, *Parasutterella* and to the family *Ruminococcaecae* in comparison to control group (Moitinho-Silva et al., 2021). The physiological stress like military training influences the intestinal permeability and modified the gut microbiota composition; moreover Shannon and chao1 index showed an increasing trend (Karl et al., 2017). A clinical trial on cross-country runners which involved them in performing different treadmill exercises on different intensities, the results depict that the performance of an individual exercise bout, in cross-country non-professional athletes, shows significant changes in the microbiota, in serum and faecal metabolome, which may have the health implications (Kulecka et al., 2020). A 6-month cohort study on male marathon and triathlon involving them in regular aerobic exercise, complimented with twice weekly resistance training and observed alterations in gut microbiota and physiologically relevant metabolites due to sustained fitness exercises (Barton et al., 2018). The systematic review shows that the microbiota can be affected by the level of physical intensity, with implications for bacterial abundance and diversity indices. These findings have implications for the physiological and metabolic system. Our meta-analysis indicates that the Firmicutes/Bacteroidetes ratio tends to increase in relation to levels of physical activity intensity. This suggests that Firmicutes may play a specific role in adaptation to higher levels of physical activity as shown in Figure 14.

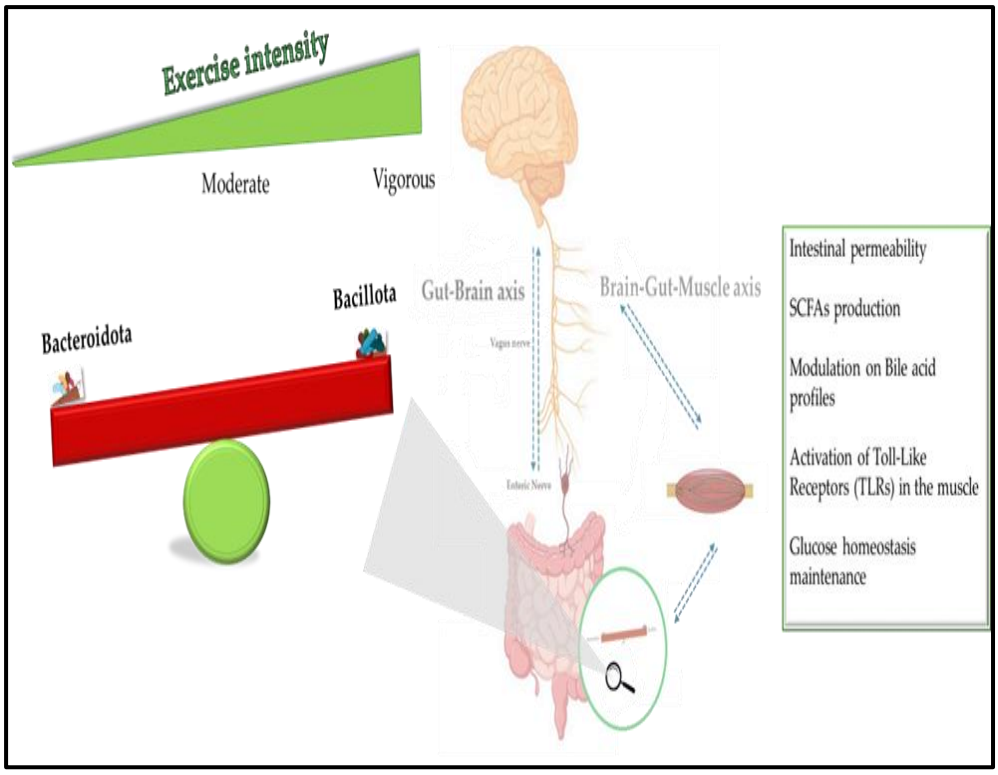


Figure 14. Variation in the *Firmicutes/Bacteoidota* ratio can occur based on physical intensity training (Ghaffar et al.,2024)

5.1.2 Narrative review: Impact of intermittent fasting on wellbeing, nutrition and physical performance in different sports

A total of 422 records were identified through database searches (Scopus, PubMed, and Google Scholar). After initial screening to remove duplicates and review papers, 92 articles remained. Full-text assessment further reduced this to 57 eligible studies, meaning 365 records were excluded by this stage. Following a second eligibility review of the 57 full-text articles, 32 studies were ultimately included in the review as shown in figure 15. At the final stage, studies were excluded if: (1) the participants were professional athletes, (2) an appropriate fasting-related nutritional strategy was not described, or (3) the intervention did not comply with intermittent Ramadan fasting rules.

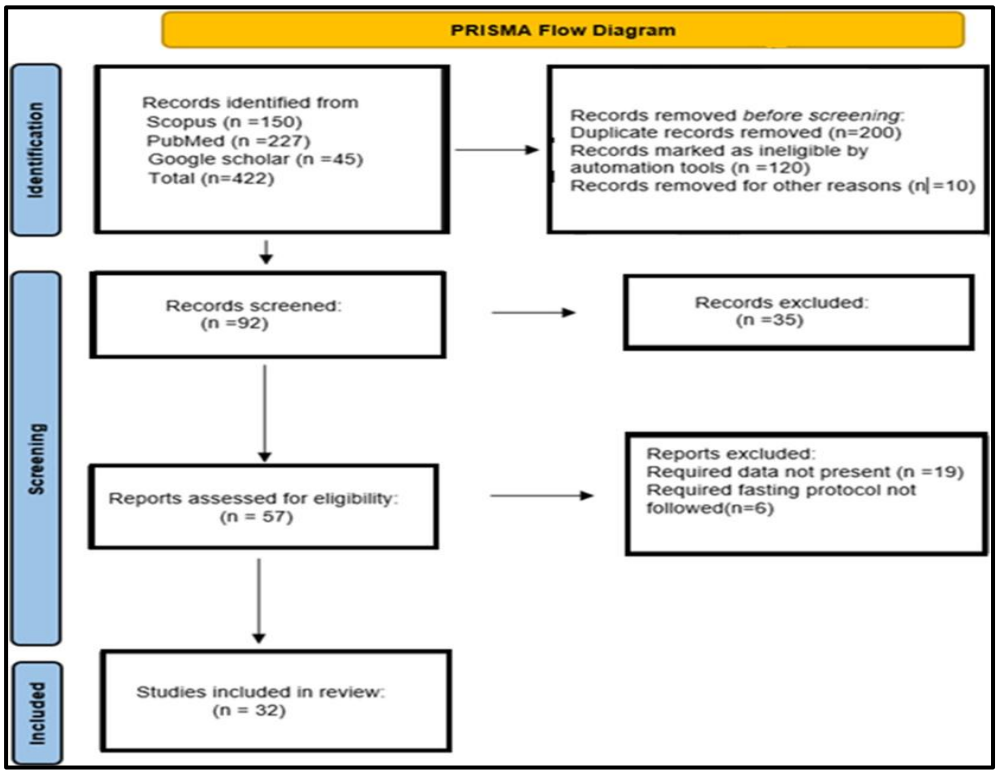


Figure 15. PRISMA flow diagram following the PRISMA guidelines (Ghaffar et al.,2025)

Across the 32 included studies, participant sample sizes ranged from 8 to 117, and the populations included adolescents and adults approximately 17 to 40 years. The included papers covered a range of multi-strategy approaches. Most studies were conducted in Muslim-majority countries, with additional studies carried out in European and other countries, including Tunisia, Singapore, Italy, the United States, Algeria, Germany, Lebanon, Spain, Greece, Malaysia, Yemen, the United Kingdom, Iran, Morocco, Kazakhstan, Portugal, and Turkey. The studies examined multiple sports and activity categories. This included endurance running, soccer, handball, basketball, football, tennis, karate, judo, archery, swimming, badminton, volleyball, kickboxing, and physically active non-athlete populations.

Table 4. Division of sports categories regarding type of sport

Sports Categorie	Type of sport
Ramadan fasting in aerobic and endurance athletes	Endurance training, Running, Sprint running
Ramadan fasting in Martial art	Karate, Archer, Judo, Kickboxers
Ramadan fasting in competitive sports	Handball, basketball, volleyball
Ramadan fasting in indoor games	Tennis, Badminton
Ramadan fasting in football & soccer players	Football, soccer
Ramadan fasting in swimmers	swimming

The Web of Science database contains 201 records related to Ramadan fasting and sport. Figure 16 illustrates publication trends over the past decade (2014 to May 2024), showing the number of papers published each year. Overall, the trend demonstrates a progressive increase in publications from 2014 onwards, indicating growing research interest in the effects of Ramadan fasting on sports and physical activity.

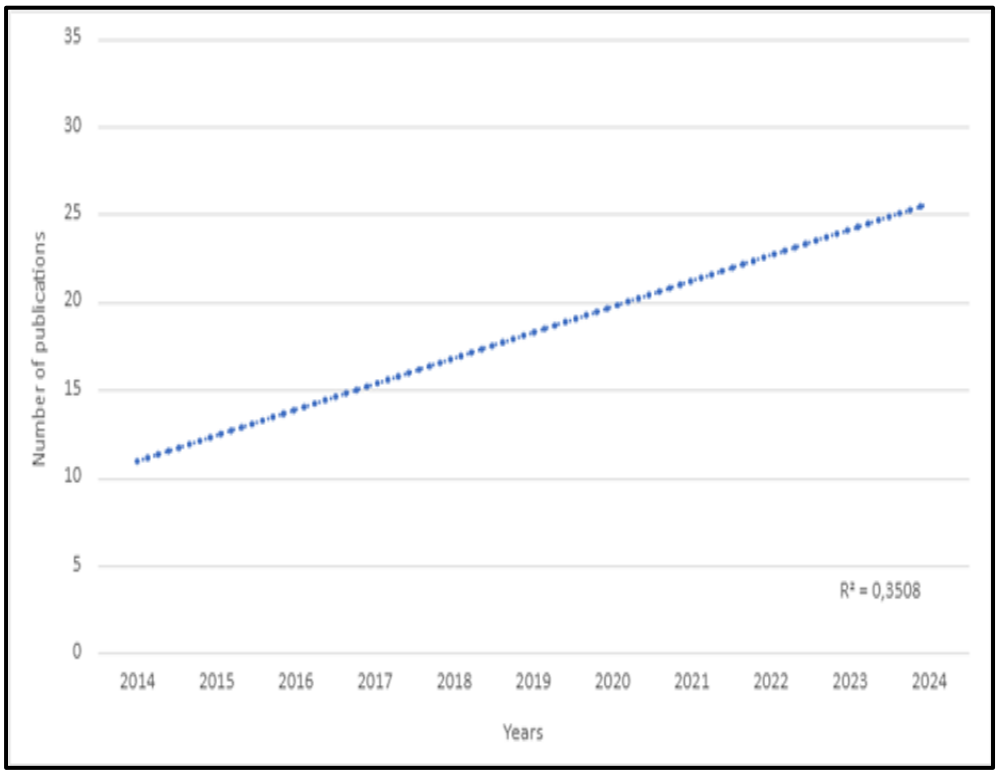


Figure 16. Yearly publication trend. The number of publications about Ramadan fasting in sports increases in the last ten years. (Ghaffar et al.,2025)

The analysis for Ramadan fasting in sports, keyword frequencies were analysed using the Web of Science database. Figure 17 presents the keyword frequency distribution. In total, 2,644 distinct research keywords were retrieved, providing a detailed overview of the thematic

landscape of this field. The most frequently occurring keywords were “Human,” “Physical activity,” “Ramadan fasting,” and “Fasting.”

A country map (Figure 18) was created to visualise the leading countries publishing on Ramadan fasting in sports. Across the analysed records, publications were attributed to 450 countries. The top ten countries by publication output were the USA, Qatar, England, Iran, France, Saudi Arabia, the United Arab Emirates, Italy, Germany, and Canada. The USA was the highest contributing country, followed by Qatar and England in second and third place, respectively. Overall, the distribution indicates that research on Ramadan fasting in sports has gained global visibility over time, with particularly strong representation from both developed countries and Islamic countries.

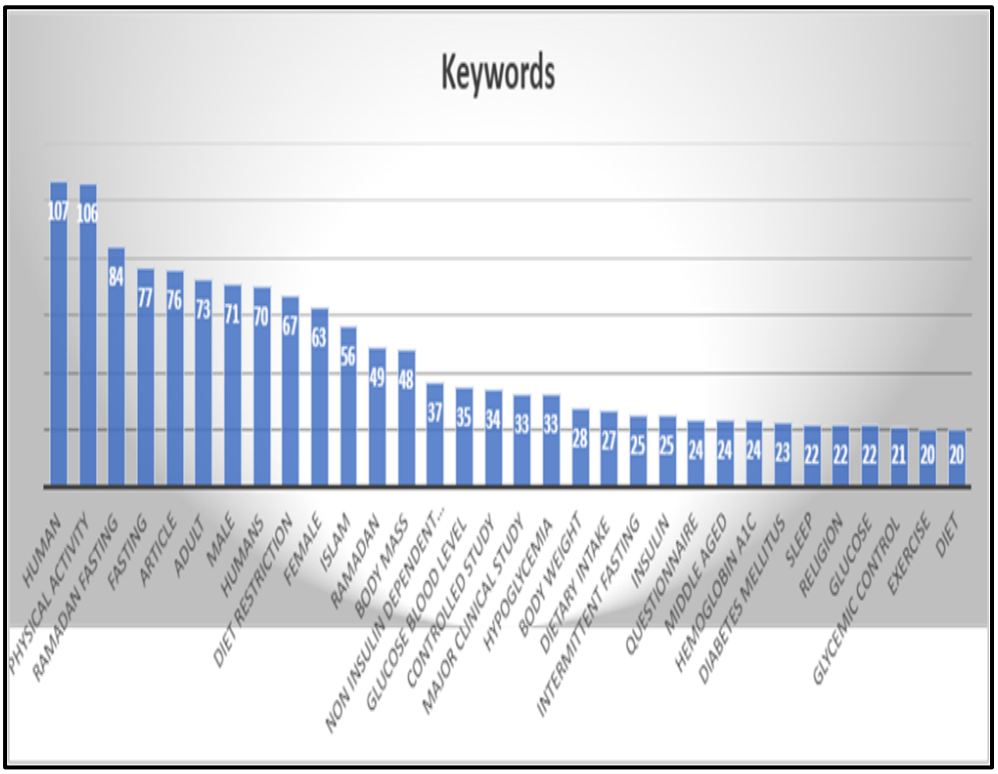


Figure 17. Keyword trend. The number of Keywords about Ramadan fasting, and Physical activity shows highest trend (Ghaffar et al.,2025)

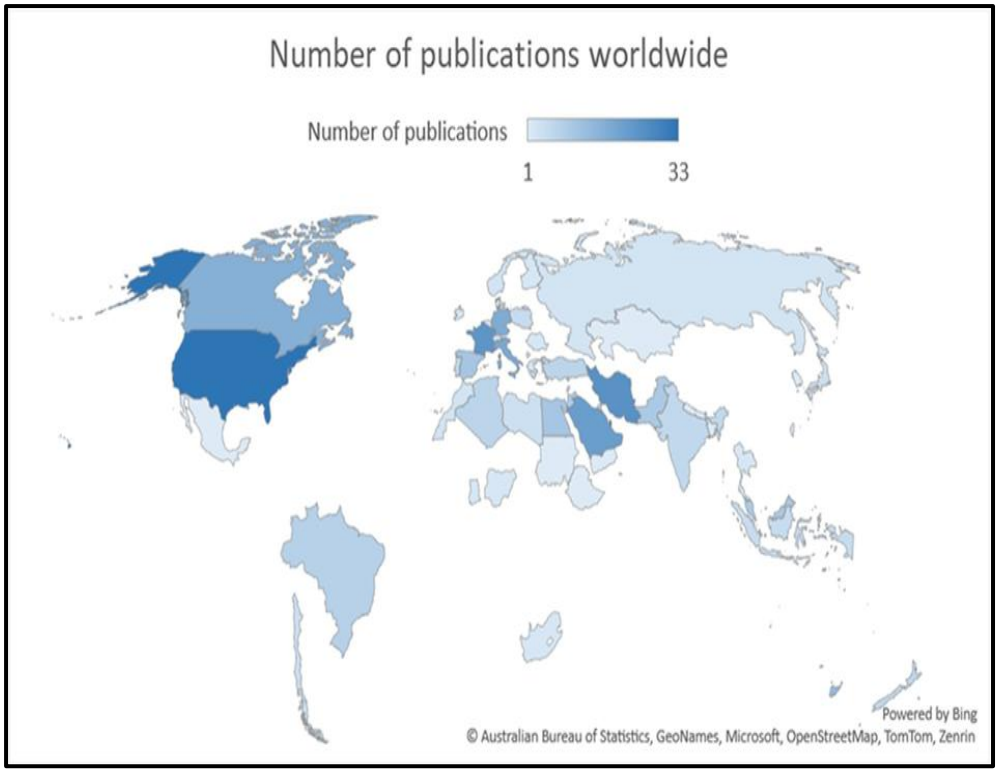


Figure 18. Publications trend Worldwide. The publication trend worldwide is highest in the USA. (Ghaffar et al.,2025)

The characteristics of the articles included in the review have been enclosed in the following table (Table 5).

Table 5. Characteristics of original studies and main results related to Ramadan fasting in different sports. ND= Not described

Author [ref]	Year	Country	Population	No of participants	AGE	Time of data collection	Tests Used for assessment physical performance
(Lipert et al., 2021)	2021	Lebonan	Healthy athletes' endurance training in form of running	32 healthy male	17-40 years	two weeks before Ramadan and during Ramadan (third week)	Copper test (Covered distance in meters + VO2 max (mlO2/kg/min)) Step test (heart rate at rest per minute & heart rate after the step test per minute)
(Zarrouk et al., 2013)	2016	Tunisia	karate athletes (black belt 1st Dan and regularly engaged in 2 h a day, 5 days a week for at least 3 years)	8 male	17.2±0.5 years	The first study session (S1) was performed one week before Ramadan, the second (S2) was performed at the end of the first week of Ramadan, and the third (S3) was performed at the end of the last week of Ramadan	simple reaction time (SRT) choice reaction time (CRT), SRTand CRT were evaluated at rest and just after neuro muscular fatiguing exercise

(Fashi et al.)	2021	spain	Athlete	12 male	23.2± 1.3 years	3 weeks after the beginning of Ramadan fasting and 2 weeks after the end Ramadan fasting.	Muscle function, rating of perceived exertion (RPE), isokinetic knee extension, extension peak torque, flexion, extension average power, flexion average power peak torque, extension total work
(Bougue rra et al., 2017)	2017	Tunisia	Highly trained endurance athletes (middle- and long-distance Runners)	24 male	28.5 ± 10.6 years)	Before, mid and after Ramadan	Running time calculated in morning, afternoon and in evening for running
(Amjad et al., 2024)	2023	Unites states, Arizona	College student athletes	12 (4 women and 8 men)	19.9±1. 4 years	During whole Ramadan month	consensual qualitative research method
(Havenet idis, 2015)	2015	Greece	Running endurance athletes (20m shuttle run test)	20 male	21.7± 2.0	Periodization training before, during and after Ramadan	Training loads from various types of exercises, ratings of perceived exertions (RPE), total quality recovery (TQR) and heart rates
(Al-Nawaise h et al., 2021)	2021	United states	Distance runners	15 male	23.9 ± 3.1 years;	pre-Ramadan and the last week of Ramadan	graded exercise test on the treadmill, VO2, Heart Rate, time to exhaustion, RPE, and running speed
(Graja et al., 2021)	2020	Tunisia	Handball players	12 females	16.5 ± 0.5 years	One week before Ramadan, first week of Ramadan and last week of Ramadan	body mass, oral temperature (OT), Hooper index, vigilance test (VT), Epworth sleepiness scale (ESS), five jump test (5-JT), modified agility T-test (MAT) and maximal standing ball-throw velocity test (MSBVT). Running-based Anaerobic Sprint Test (RAST), RPE: Rating of perceived exertion.
(Lau et al., 2023)	2022	Malaysia	Archers	13 male	16.0±1. 53 years)	one month before Ramadan and during Ramadan.	The archery shooting performance was evaluated by the total score of 36 arrows shot from a 70 meters distance.
(Mhenni et al., 2021)	2021	Tunisia	Handball players	15 female	20.1 ± 1.5 years	One week before Ramadan and 2nd and 4th week of Ramadan	hand grip (HG), ball throwing velocity (BTV), modified agility T-test (MAT), and repeated shuttle-sprint and jump ability (RSSJA) tests
(Souissi et al., 2014)	2013	Tunisia	Judo athletes	11 male	(22.5±2. 8years	one week before RIF (BRF), during the second week (SWR) of RIF, and three weeks after RIF	dynamic balance tests, the movement time, lift-up index, and impact index during the STS test
(Bencha rif et al.)	2021	Algeria	basketball players	32(gender not defined)	24.9±1. 9 years	(Three passages during Ramadan and one passage after Ramadan	brachial muscle circumference (BMC), Body mass index

(Khemila et al., 2023)	2023	Italy	football players	11 male	19.27 ± 0.9	1 week before Ramadan (BR); the second (R2); the third (R3); the fourth (R4) week of Ramadan; and 2 weeks after Ramadan	Simple (SRT) and choice (CRT) reaction times, mental rotation test (MRT) and selective attention (SA) test were measured before and after Wingate test. Rating of perceived exertion (RPE), body composition, dietary intake, profile of mood states (POMS) and Pittsburgh Sleep Quality Index (PSQI)
(Aziz et al., 2017)	2017	Singapore	football players	14 male	21.8±2.4 years	First match the first as familiarization, the second before the Ramadan month, the third occasion during Ramadan (RAM trial), and the final session was after the Ramadan month	Rating of perceived exertion, maximal isometric volumetric contractions of knee extension, maximum contraction of hand grip
(Fekih et al., 2020)	2020	Italy	Tennis players	27 male	16.9± 0.64	(One before Ramadan and three sessions in 2nd. 3rd, 4th week of Ramadan)	MAT-Agility test
(Odeh, 2023)	2023	USA	swimmers(400 meters swam)	8(4 male & 4 female)	14-16 years old	Ramadan occurred on five consecutive days during the first week of Ramadan	Stopwatch, 10 Meter measuring tape, Dynamometer (100kg max), iPad, using "Reaction time "application, Digital sphygmomanometer
(Özbay et al., 2024)	2024	Tunisia	Athletes (Sprint players)	14 male	22.4 ± 1.8 years	Start of Ramadan and day 1, 2nd and 3rd of Ramadan	oxidative, lactic and lactic metabolic pathways were quantified using the PCr-LA-O2 method
(Bouzid et al., 2019)	2019	Germany	soccer players	8(gender not defined)	21.0 ± 0.4 years	1 week before (BR) and during the fourth week of Ramadan (End-R	squat jump, countermovement jump, maximal voluntary contraction, and 20 m sprint, and creatine kinase, uric acid, and subjective ratings (feelings scale, quality of sleep, fatigue, muscle soreness and stress) were assessed at baseline and 0, 24, 48, and 72 h following LISTmod.
(Hajji et al.)	2023	Yemen	football players	108 male	25.54± 4.41 years	one week before Ramadan, during the last week of Ramadan, and one week after Ramadan	Arabic version of the Inventory of Coping Strategies for Competitive Sport (ICSCS) scale in each session
(Carling & Lugier, 2019)	2019	United Kingdom	Soccer players	12(gender not defined)	26.0 ± 4.0 years	experiment conducted in start of Ramadan at different intensity games	Global Positioning System device (GPS) sampling at 10 HZ

(Bougrine et al., 2023)	2023	Germany	Handball players	19 female	16.8± 0.4 y	ten days prior to Ramadan (R 10), the final ten days of Ramadan (R) including both Early Suhoor R(ES) and Late Suhoor R(LS) conditions, and the ten days immediately following Ramadan (R +10)	Countermovement Jumps Test (CMJ), Modified Agility t-Test (MATT), Repeated Sprint Ability (RSA), and Rating of Perceived Exertion (RPE)
(Hakoumı et al., 2018)	2017	Algeria	Athletes (race of 3000 meters)	13 male	21 to 33 years	11 days before the period of Ramadan, 25th day during the fast period, and 119th day after the fast of Ramadan	chronometric performances related to the manifestation athletes endurance capacity, intermediate distance which permit to note and detect eventual physiological variations. Axillary temperature measurement
(Ar et al., 2020)	2019	Singapore	Highly trained bedminton players	10 male	22.8 ± 3.4 y	Before Ramadan and two weeks after start of Ramadan	Questionnaire, Blood glucose test, Lactate test, Assessment of perceived exertion (RPE), using Borg's categorical scale of 1—10
(Maneshi et al.)	2023	Iran	Volleyball Players	49 female	Fasting group(29±7.3) Nonfasting group(30±5.1)	Before Ramadan, during Ramadan and 2 weeks after Ramadan recovering period	hand strength, muscle power [Sargent's jump and long jump], reaction time, service skill
(El-Jaziz & Lotfi)	2023	Morocco	Athletes	117(72 females , 45 males)	16.86 ± 1.07 years)	Before Ramadan, 2nd week of Ramadan, 4th week of Ramadan and last days of Ramadan	20-m shuttle-run test.
(Fekih et al., 2020)	2020	Italy	Tennis players	38 male	16.9± 0.6	Before Ramadan, end of first week of Ramadan, end of second and 4th week of Ramadan	A type of radar gun (sr3800-fp Radar Vitesse Pistolet) was located behind the player, to record the speed of the ball during the execute
(Bezuglov et al., 2021)	2021	Kazakhstan	Elite professional adult soccer players	26(gender not defined)	Group 1(24.0 2.8 years) Group 2 (e 26.0 4.4 years.)	Before Ramadan and third week of Ramadan	Instant system for measuring performance
(Brini et al., 2021)	2021	Portugal	basketball players	24 male	Group 1(25.32 ± 2.56)Group 2 (24.85± 1.55)	During whole Ramadan but changes accessed during first and fourth week of Ramadan	body composition, sleep habits, and ratings of perceived exertion (RPE) measured
(Yagin et al., 2022)	2022	Turkey	Kickboxers	10(gender not defined)	21.2±1.61	the last ten days of Ramadan (DR) and 3weeksafter Ramadan	Running-Based Anaerobic Sprint Test (RAST)
(Brini et al., 2023)	2023	Tunisia	basketball players	28 male	Group 1 (26.2± 2.1) Group 2 (25.8±	First and fourth week of Ramadan	internal load (weekly session rating of perceived exertion (s-RPE), heartrate (HR)), dietary intake, body composition, sleep

					2.1)		quality (PSQI survey), well-being indices questionnaire (sleep, fatigue, stress, delayed onset of muscle soreness (DOMS)), and recovery state with the Total Quality Recovery (TQR) questionnaire.
(Boukhris et al., 2019)	2019	Tunisia	Physically active individuals	12 male	21.9±2.4yrs	15-days BR, on the first (FR) and last 10-days of Ramadan (ER) and 10-days (AR10) and20-days (AR20) after Ramadan.	5 jump test evaluation
(Aziz et al., 2018)	2017	Singapore	soccer player	13 male	20.1 ± 0.9 y	Two matches before the start of Ramadan and two matches in last week of Ramadan	~80g GPS device that sampledat 5Hz (Minimax 2.5; Catapult Innovations, Melbourne, Australia) and a HR monitor strap (T-31, Polar Oy, Kempele, Finland) housed in a harness secured in the upper back.

Analysis of the results of the studies included in the systematic review showed that the intermittent Ramadan Fasting (IRF) negatively effects the physical performance, players coping behavior, nutritional status and fatigue capacities in different sports, although, its prevalence impact is more negative in Handball, Basketball, Volleyball, Football and swimmers although the impact is less negative for Tennis, Badminton, martial arts and endurance sports. Moreover, increasing carbohydrate intake, reducing training session duration, increasing sleep time or adding other recovery methods could improve fatigue, performance behavior and reduce the increased muscle soreness experienced with increased game frequency crossing RIF.

5.1.3 Systematic review: Sex related gut microbiota differences in health and disease

A total of 13,205 records were identified through database searches: Scopus (3,272), PubMed (4,520), and Google Scholar (5,413). After the initial screening to remove duplicates and review papers, 11,961 records remained. Following full-text assessment excluding studies involving unhealthy populations, gastrointestinal disease, and articles not meeting the inclusion criteria 135 articles were retained, meaning 13,067 records had been excluded at that stage.

In the second eligibility screening, 100 full-text articles were evaluated, and 24 studies were ultimately included in the review (Figure 19). At this final stage, studies were excluded if: (1) they were not conducted in participants of different sexes, (2) sex differences were not assessed under comparable conditions, or (3) participants did not meet the selection criteria, particularly due to gastrointestinal disease or other indicators of an unhealthy population. This systematic

review encompassed 24 research articles, including 15 human studies. The twenty four selected studies were published between 2006 and 2024 and show a very stable trend. Most studies were conducted in China and remaining in other countries including the USA, Netherlands, Spain, Japan, Italy, Isreal, Hungary, Germany, Canada and Korea. The mean age of participants is 53.5 years. All studies utilizing NGS, including shotgun sequencing or 16S rRNA amplicon sequencing, and independent of culture-based techniques.

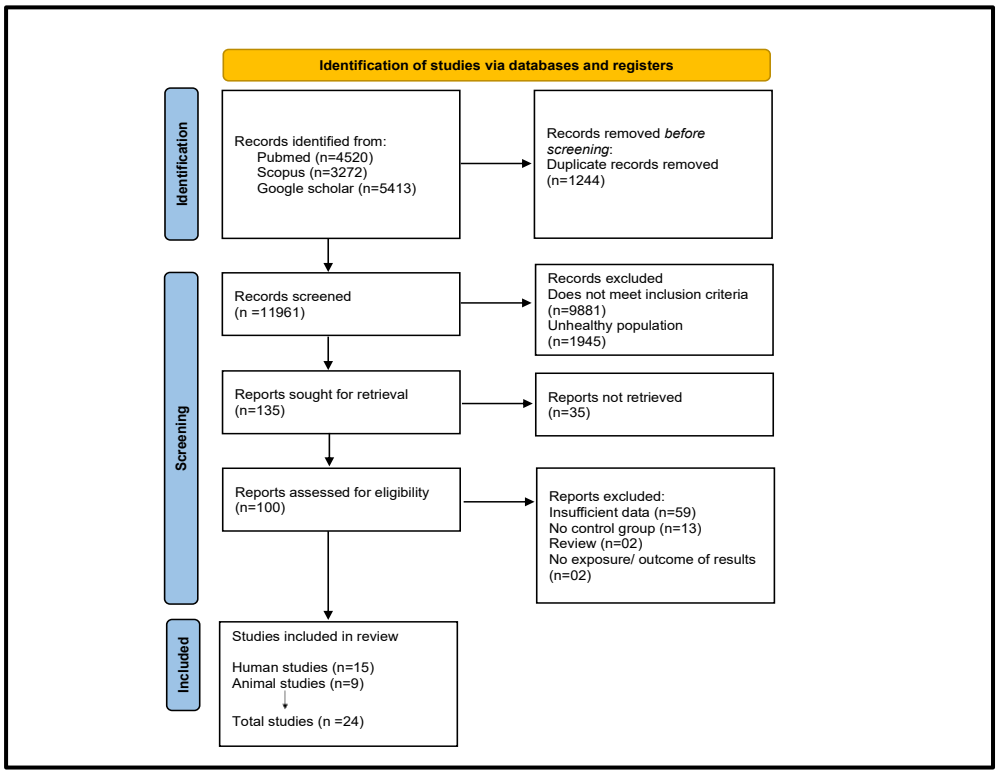


Figure 19. PRISMA flow diagram following the PRISMA guidelines (Ghaffar et al.,2025)

The studies enclosed in the systematic review are divided into two groups, one group contains all the human studies (Table 6) and the other group includes all the animal studies (Table 7).

Table 6: Characteristics of human Studies regarding sex differences in gut microbiota

Author, Year, Country, Ref	Participant	Male-Female ratio M: F	Age	Study Method	Outcomes
(Mueller et al., 2006)	Total 230	N/A	Adult group (aged 20 to 50 years), Elderly group (>60 years of age)	16S rRNA-targeted /fluorescence *i nsitu* hybridization analysis	At the species level, no sex differences were observed for *Bacteroides vulgatus* and *Bacteroides putredinis*. There was no detectable sex effect for any of the other microbial groups. ↑*Bacteroides-Prevotella* group in males than in females.

(Feng et al., 2014)	Total 108 46 Male, 62 Female	45:55	42.8–51.0 years	16S rRNA gene sequencing	The fecal *F. prausnitzii* amount was not significantly different between obese and lean subjects. Men possessed a significantly lower level of fecal *F. prausnitzii* than women, and the significant and positive correlation of fecal *F. prausnitzii* quantity with fasting glucose level was observed in men, not in women
(Ding & Schloss, 2014)	Total 300 149 males, 151 females	50:50	18–40 years	16S rRNA gene sequencing	Sex was associated with community types (fecal, oral, vaginal, skin) identified in the stool. Males were 3 times more likely than females to harbor stool community type. Stool community in males had low levels of *Bacteroides* but higher *Prevotella.*
(Dominianni et al., 2015)	Total 82 51 males, 31 females	62:38	30–83 years	16S rRNA gene sequencing	Sex was significantly associated with the overall gut microbiome composition. ↓*Bacteroidetes* in females. The relationship between BMI and gut microbiota composition was significant in females, but not in males. ↓Shannon-diversity indices for overweight and obese subjects compared with normal-weight subjects in females, but no difference in males.
(Singh & Manning, 2016)	Total 200 141 male, 126 female	53:47	1 to 90 years	16S rRNA gene sequencing	Sex was significantly associated with the overall gut microbiota composition: At the genus level, ↑*Bacteroides* in females, ↑*Escherichia* in males. There was no sex difference in gut microbiota in healthy individuals. There was a significant difference between sexes in individuals with enteric infection: ↑*Enterobacteriaceae* among 11 different features in males, ↑*Bacteroidaceae* among 43 different features in females.
(Haro et al., 2016)	Total 75	52:48	Females 60.31±1.40, males 61.15±1.27	16S rRNA gene sequencing/MiSeq (Illumina)	The microbiota diversity and overall community composition were not significantly different between sexes. ↑*Veillonella* and *Methanobrevibacter* at the genus level, *Bacteroides plebeius* and *Coprococcus catus* at the species level in males. ↑*Bilophila* at the genus level, *Bacteroides caccae* at the species level in females. There were no differences at the phylum level and in the F/B ratio between sexes when considered independently of the BMI.The F/B ratio was higher when BMI ≤33 kg/m^2 and lower when BMI >33 kg/m^2 in males compared with females. ↓*Bacteroides* at the genus level in males when BMI > 33 kg/m^2.
(Borgo et al., 2018)	Total 40	50:50	males 48.7±10.2, females 51.7±8.3,	16S rRNA gene sequencing/MiSeq (Illumina)	There was no difference in lumen-associated microbiota between the sexes. ↑α-diversity in MAM in females. ↑*Actinobacteria* and *Lactobacillales* in MAM of females. ↑*Bifidobacterium spp*. and *Streptococcaceae*. ↓*Veillonellaceae* and unclassified *Clostridia* in MAM of females.
(Gao et al., 2018)	Total 551	47:53	males 37.8±17.3, females 35.6±14.3	16S rRNA gene sequencing	There was no significant overall difference in gut microbiota between the sexes. ↑α-diversity in females. Regardless of the BMI, there were no significant differences at the phylum, class, order, or family levels between the sexes, but ↑*Ruminococcus* at the genus level in females. In obese subjects, at the genus level, ↑*Bifidobacterium*, *Coprococcus*, and *Dialister* in females, ↓*Phascolarctobacterium* in females, ↑*Fusobacterium* in males. In underweight subjects, at the genus level, ↓*Sutterella* in males.

(Sinha et al., 2019)	Total 1135	42:58	18-81 years	Shotgun sequencing	Sex was significantly associated with the overall gut microbiota composition, 12 microbial species, and 43 metabolic pathways. ↑*Akkermansia muciniphila* in females. ↑Richness of antibiotic resistance genes in females.
(Takagi et al., 2019)	Total 277	50:50	20-89 years	16S rRNA gene sequencing/MiSeq (Illumina)	There was no significant difference in α-diversity between males and females. ↑Genera *Prevotella*, *Megamonas*, *Fusobacterium*, and *Megasphaera* in males. ↑Genera *Bifidobacterium*, *Ruminococcus*, and *Akkermansia* in females.
(Shin et al., 2019)	Total 57 31 males, 26 females	55:45	Male age 37.45 ± 1.91 Female age 46.15 ± 2.26	16S rRNA gene sequencing	Men and women in the High group harbored more diverse gut microbial communities than others. In men, the abundance of *Acinetobacter*, *Dorea*, *Ruminococcus*, and *Megamonas* correlated significantly with testosterone levels. Women in the High group have more *Bacteroidetes* and less *Firmicutes* phyla than those in the Low group. Genera *Slackia* and *Butyricimonas* were significantly correlated with estradiol levels
(Cuesta-Zuluaga et al., 2019)	Total 8,924 Male female no not specified	NA	20 to 69 years	16S rRNA gene sequencing	Young adult women had consistently higher alpha diversity and predicted microbiota age than men in the US, UK, and Colombian cohorts. Specific taxa such as *Ruminococcaceae, Lachnospiraceae*, and *Akkermansia* were associated with higher diversity and enriched in females. These sex-based differences were not observed in the Chinese cohort, suggesting possible geographic or lifestyle influences on microbiome aging. Overall, the findings highlight age- and sex-dependent patterns in gut microbiome composition.
(Kalabiska et al., 2023)	Total 124 62 males, 62 females	50:50	16-20 years	16S rRNA gene sequencing	Predominance of *Streptococcus* and *Veillonella* genera was visually greater in females vs. males and in water polo players vs. controls. Compared to females, males had significantly higher relative abundance of the following genera: *Atopobium*, *Pravotella* very large effect size. Compared to females, males had significantly lower relative abundance of the genera: *Fusobacterium*, *Gemella*, and *Streptococcus*. Compared to the control group, water polo players had significantly higher relative abundance of the genus *Veillonella* and significantly lower relative abundance of the genus *Gemella*.
(Ghazal, 2024)	Total 44 22 males, 22 females	51:49	Athlete group 21.55 ± 2.42 years Control group 21.27 ± 2.51 years	16S rRNA gene sequencing	Female Elite Athletes revealed notable components such as *Bacteroides* (28.48%), *Prevotella* (21.86%), *Megamonas* (11.43%),*Megasphaera* (6.01%), and *Lachnospira* (3.75%). Conversely, male Elite Athletes exhibited a distinct microbial composition, with *Prevotella* (27.00%), *Bacteroides* (15.82%), *Megamonas* (11.55%), *Faecalibacterium* (9.63%), and *Alloprevotella* (5.77%) as the primary genera. Differences in alpha and beta diversity measures were observed between male and female Elite Athletes, indicating variations in the overall diversity of their gut microbiota
(Shalmon et al., 2024)	Total 40 22 males, 18 females	55:45	Athlete group 43 ± 6.5 years, Control group 41 ± 7.4 years	16S rRNA gene sequencing	No difference in alpha diversity was observed between males and females in any of the groups. Female runners showed a lower abundance of *Enterococcus*, a higher abundance of *Lactobacillus*, and a higher abundance of *Methanosphaera* than male runners, while male runners exhibit lower abundance of *Enterobacteriaceace* & *Lactobacillus ruminis* while higher abundance of *methanosphaera*.

Table 7: Characteristics of animal Studies regarding sex differences in gut microbiota

Author, Year, Ref	Participant	Male Female ratio M: F	Age	Study method	Outcomes
(Yurkovetskiy et al., 2013)	Total 39 mice	50:50	4 to 13 weeks	Shotgun sequencing	The abundance of *Porphyromonadaceae, Veillonellaceae, Kineosporiaceae, Peptococcaceae, Enterobacteriaceae, Lactobacillaceae, Cytophagaceae, Peptostreptococcaceae, and Bacteroidaceae* at the family level was higher in the male mice than in the female mice
(Markle et al., 2013)	Total 255 mice 123 males, 132 females	48:50	7 to 14 weeks	16S rRNA gene amplicon sequencing	Intestinal communities were distinct among male and female mice. Sex-specific differences in the intestinal community were most apparent in adult mice. Overall, the dominant genera in males were *Bilophila , Alistipes, Ruminococcus, Peptococcus*, and *Deltaproteobacteria*, whereas in females the dominant genera were *Lachnospiraceae*, *Lachno I.S.*, *Coprococcus 1*, and *Blautia*.
(Org et al., 2016)	Total 689 mice 341 females 348 males	49:51	8 weeks	16S rRNA gene sequencing	Phylum *Actinobacteria* (0.81 ± 0.19 males; 0.18 ± 0.1 females) and *Tenericutes* (1.08 ± 0.22 males; 0.35 ± 0.12 females) were more abundant in male than female mice. Genera with higher abundance in males as compared to female mice included *Allobaculum*, *Anaeroplasma*, and *Erwinia*, whereas *SMB53* from family *Colstridiaceae* and 3 members of family *Lachnospiraceae* (*Dorea, Coprococcus and Ruminococcus*) were more abundant in female mice
(Fransen et al., 2017)	Total 68 mice	49:51	7- to 10-week	16S rRNA gene sequencing	Conventional females had higher abundance of *Desulfovibrionaceae*, *Lactobacillaceae*, and *Verrucomicrobiaceae*, whereas conventional males had higher abundance of *Ruminococcaceae* and *Rikenellaceae*. Female recipients had a higher abundance of *Lactobacillaceae* and male recipients had a higher abundance of *Desulfovibrionaceae*, *Ruminococcaceae*, and *Porphyromonadaceae*. Four weeks after transfer, most differences were still dependent on the sexof the host, but there were also some donor-dependent differences. For example, lactobacilli were still more abundant in female recipients, whereas *Akkermansia* and *Prevotellaceae* were more abundant in male recipients. In these mice, *Rikenella*, *Lachnospiraceae*, and *Desulfovibrionaceae* were increased after transfer of male microbiota, but *Prevotellaceae* was increased after transfer of the female microbiota.
(Elderman et al., 2018)	Total 10 mice, 5 males 5 females	50:50	8 weeks	16S rRNA gene sequencing	*Lactobacillus plantarum* and *Bacteroides distasonis* were enriched in B6 females as compared to B6 males, while *Bifidobacterium* was enriched in BALB/c females as compared to BALB/c males. Overall, males show less diversity and richness as compared to females. Sex explained 11.6% of the variance in microbiota composition
(Zhao et al., 2019)	Total 20 deer 10 males 10 females	50:50	Juvenile 1–2-year Adults 4–10 years	16S rRNA gene sequencing	At the phylum level, the *Firmicutes* were most abundant in JMF (71.15%) followed by AFF (58.66%), JFF (55.90%), and AMF (55.66%). On the contrary, the *Proteobacteria* was abundant in AMF (28.67%) followed by JFF (19.43%), AFF (17.45), and JMF (10.35%). The enrichment of *Bacteroidetes* was observed in AFF (20.65%), JFF (19.17%), JMF (17.02%), and AMF (12.59%). Amongst all groups, the presence of *Pseudomonas* was recorded as higher in AMF (12.90%) and AFF (12.31%) in average
(He et al., 2019)	Total 293 pigs 108 female gilts, 185 male boars	58:42	80,120, 140 days	16S rRNA gene sequencing	*Veillonellaceae, Roseburia, Bulleidia*, and *Escherichia,* which showed the higher abundance in boars, and *Treponema* and *Bacteroides* which were over-represented in gilts. Castration significantly shifted the fecal microbiota composition of the boars toward that of gilts. At the family level, eight families were identified with significantly different abundances between entire boars and gilts in the Duroc population. *Veillonellaceae* and *Enterobacteriaceae* were more abundant in entire boars. However, *Spirochaetaceae* and *Bacteroidaceae* were significantly enriched in gilts. At the genus level, a total of 8 bacterial genera displayed differing enrichments between entire boars and gilts. For example, *Bulleidia* and *Escherichia* were of higher abundance

					in entire boars, whilst *Treponema* and *Bacteroides* were significantly enriched in gilts.
(Gan et al., 2023)	Total 42 mice 18 female, 24 male	4:3	Age not defined	16S rRNA gene sequencing	*Firmicutes* (33.8% for male and 49% for female), *Bacteroidetes* (46.3% for male and 33.8% for female), *Proteobacteria* (4.7% for male and 8.8% for female), and *Actinobacteriota* (3.2% for male and 2.5% for female) were the main enrichment of the microbial community, and the relative abundance of other bacteria was lower than 1%.
(Sisk-Hackworth et al., 2023)	Total 77 mice 37 female, 40 male	52:48	10 weeks old.	16S rRNA gene sequencing	*Males had higher abundance of Alistipes and Rikenellaceae, while females showed enrichment of Lactobacillus and Erysipelotrichaceae*

The impact of sex differences in humans based on individual taxa in gut microbiota in Table 8 and all the specific and dominant genera in the animal studies as a comparison between males and females are present in Table 8.

Table 8: Comparison of gut microbial diversity in males and females in animals studies

Reference	Microbial Diversity in Male	Microbial Diversity in Female
(Mueller et al., 2006)	↑ *Bacteroidetes* ↑*Prevotella*	↓*Bacteroidetes* ↓*Prevotella*
(Feng et al., 2014)	↓*F.prausnitzzi*	↑*F.prausnitzzi*
(Ding & Schloss, 2014)	↓*Bacteroides* ↑*Prevotella*	↑*Bacteroides* ↓*Prevotella* ↓*Ruminococcaece*
(Dominianni et al., 2015)	↑*Bacteroidetes* ↓*Firmicutes*	↓*Bacteroidetes* ↑*Firmicutes* ↑*Actinobacteria*
(Singh & Manning, 2016)	↑*Escherichia* ↑*Paraprevotella* ↓*Enterobacteriae* ↓*Fecalibacterium* ↓*Bacteroides*	↑*Bacteroides* ↑*Dialister* ↑*Lactobacillus* ↑*Lactobacilaceae* ↑*Comamonadaceae* ↑*Comamonas* ↑*Enterobacteriaea* ↑*Fecalibacterium*
(Haro et al., 2016)	↑*Veillonella* ↑*Methanobrevibacter* ↑*Bacteroides plebius* ↑*coprococcus catus*	↑*Bilophila* ↑*Bacteroides caccae*
(Cong et al., 2016)	↑*Enterobacterilas* ↑*Pantoea* ↑*Campylobacter* ↑*Proteobacteria* ↓*Firmicutes*	↑*Firmicutes* ↑*Clostridiates* ↓*Enterobacterials* ↑*Yersinia* ↑*Formicate*
(Gao et al., 2018)	↓*Ruminococcus*	↑*Ruminococcus*
(Borgo et al., 2018)	↓*Actinobacteria* ↓*Lactobacilllus* ↓*Bifidobacterium* ↓*Streptococcaecae* ↑*Veillonellaceae* ↑*Clostridia* ↑*Gemmiger formicilis*	↑*Actinobacteria* ↑*Lactobacilllus* ↑*Bifidobacterium* ↑*Streptococcaecae* ↓*Veillonellaceae* ↓*Clostridia* ↑*Bifidobacterium adolescentis*

(Sinha et al., 2019)	↓*Akkermansia muciniphila*	↑*Akkermansia muciniphila*
(Takagi et al., 2019)	↑*Prevotella* ↑*Megamonas* ↑*Fusobacterium* ↑*Megasphera* ↓*Bifidobacterium* ↓*Ruminococcus* ↓*Akkermensia*	↑*Bifidobacterium* ↑*Ruminococcus* ↑*Akkermensia* ↓*Prevotella* ↓*Megamonas* ↓*Fusobacterium* ↓*Megasphera*
(Shin et al., 2019)	↑*Actinobacteria* ↑*Dorea* ↑*Ruminococcus* ↑*Megamonas* ↓*Bacteroidetes* ↑*Firmicutes*	↑*Bacteroidetes* ↓*Firmicutes* ↓*Actinobacteria*
(Cuesta-Zuluaga et al., 2019)	↓*Ruminococcaceae* ↓*Lachnospiraceae* ↓*Akkermansia*	↑*Ruminococcaceae* ↑*Lachnospiraceae* ↑*Akkermansia*
(Kalabiska et al., 2023)	↑*Atopobium* ↑*Pravotella* ↓*Veillonella* ↓*Fusobacteriuym* ↓*Streptococcus*	↑*Veillonella* ↑*Fusobacteriuym* ↑*Streptococcus* ↑*Gemella*
(Ghazal, 2024)	↓*Bacteroides* ↑*Prevotella* ↑*Megamonas* ↓*Megasphera* ↓*Lachnospira* ↑*Fecalibacterium* ↑*Alloprevatella*	↑*Bacteroides* ↓*Prevotella* ↓*Megamonas* ↑*Megasphera* ↑*Lachnospira* ↓*Fecalibacterium* ↓*Alloprevatella*
(Shalmon et al., 2024)	↓*Enterobacteriaceae* ↑*Methanosphaera* ↓*Lactobacillus ruminis*	↑*Methanosphaera* ↑*Mitsuokella* ↓*Enterococcus* ↑*Lactobacillus*

Table 9: Comparison of gut microbial diversity in males and females in humans studies

References	Microbial diversity in Male	Microbial diversity in Femlaes
(Yurkovetskiy et al., 2013)	↑*Porphyromonadaceae* ↑*Veillonellaceae* ↑*Kineosporiaceae* ↑*Peptococcaceae* ↑*Enterobacteriaceae* ↑*Lactobacillaceae* ↑*Cytophagaceae* ↑*Cytophagaceae* ↑*Peptostreptococcace* ↑*Bacteroidaceae*	↓*Porphyromonadaceae* ↓*Veillonellaceae* ↓*Kineosporiaceae* ↓*Peptococcaceae* ↓*Enterobacteriaceae* ↓*Lactobacillaceae* ↓*Cytophagaceae* ↓*Cytophagaceae* ↓*Peptostreptococcace* ↓*Bacteroidaceae*

(Markle et al., 2013)	↑*Bilophila* ↑*Alistipes* ↑*Ruminococcus* ↑*Peptococcus* ↑*Deltaproteobacteria*	↑*Lachnospiraceae* ↑*Lachno I.S.* ↑*Coprococcus 1* ↑*Blautia*
(Org et al., 2016)	↑*Actinobacteria* ↑*Tenericutes* ↑*Allobaculum* ↑*Anaeroplasma* ↑*Erwinia*	↑*Closatridiaceae* ↑*Lachnospiraceae* ↑*Dorea* ↑*Coproccus* ↑*Ruminococcus*
(Fransen et al., 2017)	↑*Ruminococcaceae* ↑*Alistipes* ↓*Lactobacillus* ↓*Akkermensia*	↑*Lactobacillus* ↑*Akkermensia* ↓*Ruminococcaceae* ↓*Alistipes*
(Elderman et al., 2018)	↓*Lactobacillus plantarum* ↓*Bacteroides dustasonis* ↓*Bifidobacterium*	↑*Lactobacillus plantarum* ↑*Bacteroides dustasonis* ↑*Bifidobacterium*
(Zhao et al., 2019)	Juvenile male: ↑*Firmicutes* ↑*Ruminococcaea* ↑*Dore* ↑*5-7NI5* *Adult male:* ↑*Proteobacteria*	Juvenile female: ↑*Bacteroidetes* ↑*Proteobacteria* ↑*Clostridiales* ↑*Bacteroidales* *Adult female:* ↑*Firmicutes* ↑*Bacteroidetes* ↑*Pseudomonas* ↑*Lachnospiraceae*
(He et al., 2019)	↑*Veillonella* ↑*Roseburia* ↑*Bulleidia* ↑*Escheria* ↑*Enterrobacteriace*	↑*Bacteroides* ↑*Treponema* ↑*Spirochaetaceae* ↑*Bacteroidaceae*
(Sisk-Hackworth et al., 2023)	↑*Alistipes* ↑*Rikenellaceae,*	↑ *Lactobacillus* ↑ *Erysipelotrichaceae*
(Gan et al., 2023)	↓Firmicutes ↑Bacteroidata ↓Proteobacteria ↑Actinobacteria ↓Bacteroides	↑Firmicutes ↓Bacteroidata ↑Proteobacteria ↓Actinobacteria ↑Bacteroides

As far as biological sex differences is concerned, Tables 6, 7, 8 and 9 show the compelling evidence that sex plays an important role as a biological variable affecting the gut microbiota composition in both humans and animals. Overall, evidence from both human and animal studies indicates that sex-related differences in gut microbiota are present but generally modest and strongly context dependent. In humans, early multi-centre work reported sex-associated variation in the *Bacteroides–Prevotella* group, with higher levels in males, while other targeted groups showed limited differences (Mueller et al., 2006). Later analyses of Human Microbiome

Project stool data also linked sex to distinct community patterns, including male-associated profiles with relatively lower *Bacteroides* and higher *Prevotella* (Ding & Schloss, 2014). In a large Dutch cohort, females showed higher abundance of *Akkermansia muciniphila*, yet sex explained only a small fraction of total variance in gut microbiota composition (Sinha et al., 2019). In animals, sex effects are often more pronounced: in non-obese diabetic mice, sex differences emerged more clearly after puberty, with multiple bacterial families enriched in males (Yurkovetskiy et al., 2013), and across common laboratory strains, females frequently exhibited higher diversity/richness with sex explaining a measurable proportion of community variance (Elderman et al., 2018). Additionally, broad surveys across many inbred mouse strains found that sex-associated differences vary in magnitude and direction depending on host genetic background (Org et al., 2016).

5.2 RESULTS OF REGULATION OF GUT MICROBIOTA IN RELATIONSHIP BETWEEN AIKIDO LEVELS AND MEDITATION DEPTH

The cohorts were highly homogeneous. Consistent with this, the t-test showed no significant differences in BMI, sex, age, education level, smoking status, alcohol consumption, diet, or Aikido level ($R = -0.011$, $p = 0.5$; Table 10). Sequencing of 23 samples generated a total of 1,688,571 reads (one sample was excluded because it did not meet the analysis prerequisites). Per-sample read counts ranged from 20,000 to 92,000, resulting in the identification of 471 OTUs clustered at 99% sequence identity. The socio demographic characteristics of the samples are shown in the table 10.

Table 10. Socio-demographic characteristics of the sample (n=24).

Variable	Value	Percentage
Gender		
female	9	37.5
male	15	62.5
Age		
18 – 30	1	4.2
31 – 65	20	83.3
> 65	3	12.5
Educational level		
Middle school graduation	1	4.2
High school degree	9	37.5
Bachelor's degree	1	4.2
Master's degree	9	37.5
PhD	4	16.7
Smoking		

No	16	66.7
Yes	8	33.3
Smoking frequency		
At least once/day	6	75
4-6 times/week	1	12.5
Maximum 3 times/week	1	12.5
BMI		
Normal weight	17	70.8
Overweight	6	25
Obese	1	4.2
Diet		
No diet	10	41.7
Mediterranean	8	33.3
Vegetarian	3	12.5
Requested by physician	1	4.2
Caloric restriction	1	4.2
Other	1	4.2
Alcohol consumption		
Often (> 5 times/month)	11	45.8
Sometimes (3-4 times/month)	10	41.7
Occasionally (1-2 times/month)	3	12.5
Aikido practice		
< 300 min/week	14	58.3
> 300 min/week	10	41.7
General physical activity		
Partially active	4	16.7
Physically active	20	83.3
MEDI score		
Low (0 – 60)	10	41.7
Medium (60 – 80)	7	29.2
High (80 – 120)	7	29.2
Meditation time		
< 60 min/week	13	54.2
> 60 min/week	11	45.8

5.2.1 Alpha diversity indices

Regression analyses indicated that only the meditation score was significantly and inversely associated with the Shannon diversity index ($p = 0.02$; OR = −0.08; 95% CI: −0.16 to −0.01). As shown in Figure 20A, the x-axis categories were coded as 0 (low meditation), 1 (medium), and 2 (high). In contrast, α-diversity of the practitioners' gut microbiota did not differ by Aikido level (Figure 20B). Consistently, Chao1 ($p = 0.796$) and ACE ($p = 0.886$) indices were not significantly different between group 1 (high Aikido level) and group 2 (insufficient Aikido level). Nevertheless, α-diversity tended to be lower at higher meditation levels. Moreover, training intensity was associated with significant differences in richness, as reflected by Chao1 ($p = 0.006$) and ACE ($p = 0.004$).

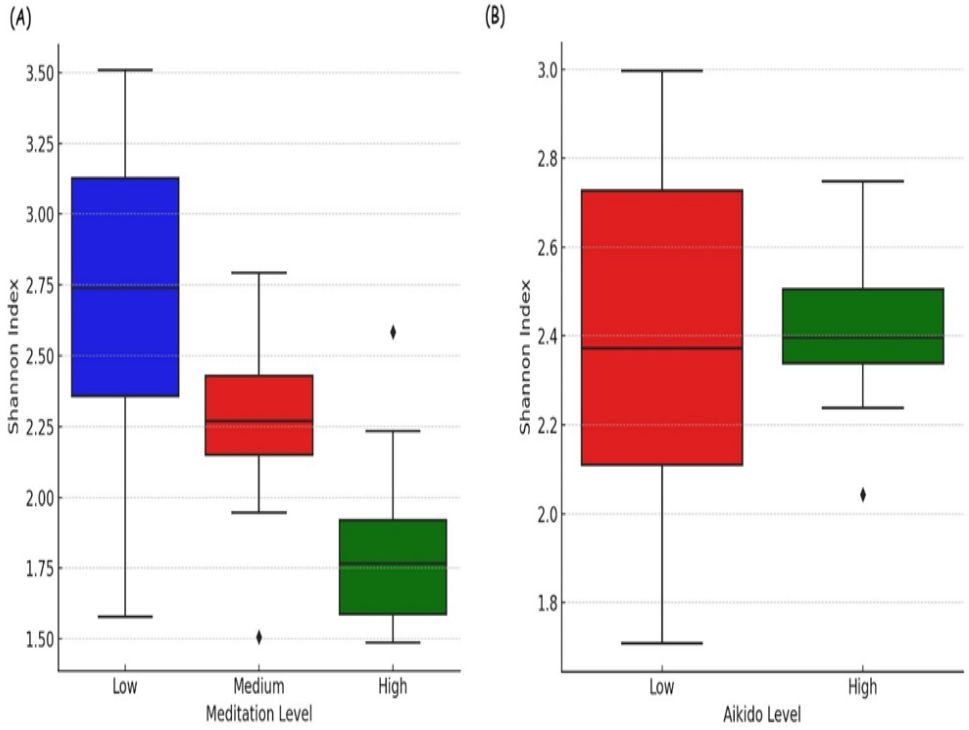


Figure 20. Box plot showing the distribution of the Shannon index in relationship between Aikido levels and Meditation depth. (Ghaffar et al.,2026)

A) Shannon value trend in three levels of Meditation. A significant reduction in alpha diversity is observed in the high meditation group, suggesting a potential compositional shift in gut microbiota associated with deeper meditative practices. B) Shannon value trend in two levels of Aikido. The Shannon index appears slightly higher in the low Aikido practice group compared to the high practice group

5.2.2 Beta Diversity and Compositional Differences (PCoA)

Beta diversity was visualized using PCoA based on Bray–Curtis dissimilarity, with samples coloured by meditation level (Figure 21). Overall, gut microbiota composition differed significantly according to Aikido practice and meditation level ($p = 0.001$), and a trend was also observed for meditation level alone ($p < 0.1$). The PCoA plot showed distinct clustering across the three meditation groups, with partial overlap between groups 0 and 1, whereas group 2 was more dispersed along the first principal coordinate. The AIKIDO variable did not produce a complete separation of samples, although directional shifts were apparent, particularly within meditation group 1, suggesting a possible interaction between physical–meditative practice and microbial community structure. These patterns were supported by ANOSIM for meditation score, which indicated a moderate but significant effect ($R = 0.350$, $p = 0.001$). The first two PCoA axes explained approximately 33.62% and 19.49% of the total variance in community composition.

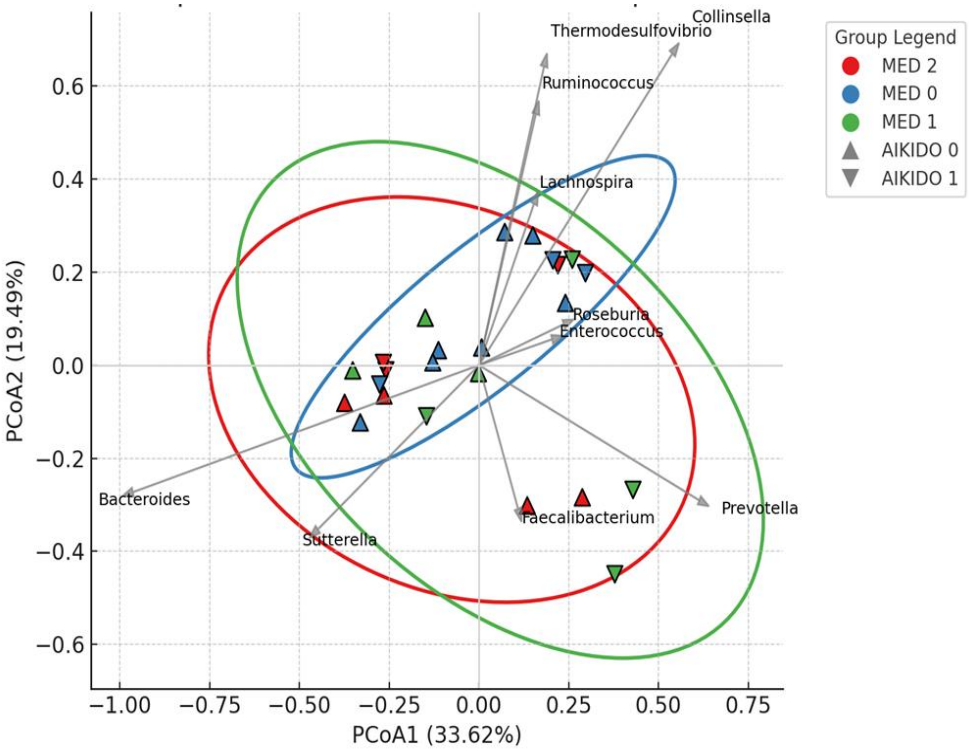


Figure 21. Principal Coordinates Analysis (PCoA) biplot based on Bray–Curtis dissimilarity of genus-level microbial profiles (Ghaffar et al.,2026)

With respect to Aikido practice, only modest compositional shifts were detected, but these were supported by ANOSIM, indicating a significant effect (R = 0.250, p = 0.001)

5.2.3 Gut Microbiota Composition differences based on meditation levels & Gender

At the phylum level, Firmicutes (53% ± 1) and Bacteroidetes (34%) dominated across groups, with lower relative contributions from Proteobacteria (6.7%), Actinobacteria (3.2%), and Verrucomicrobia (2.0%). Collectively, these five phyla accounted for more than 98% of the gut microbial community (Figure 22).

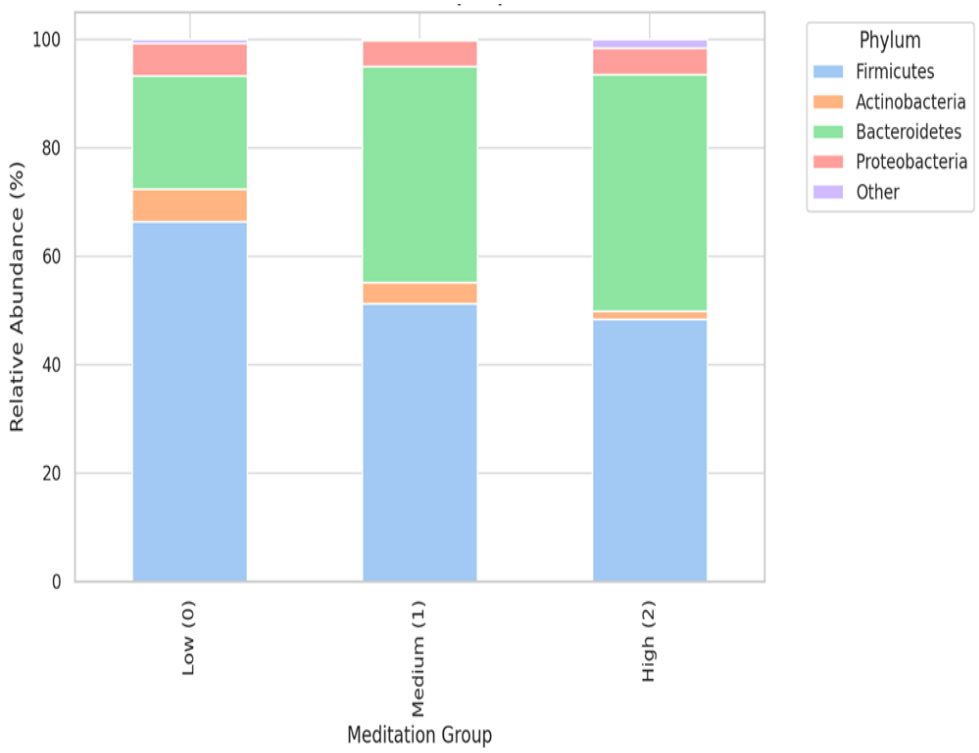


Figure 22. Normalized relative abundance of bacterial phyla across meditation levels (Ghaffar et al.,2026)

When microbial composition was stratified by meditation level, clear phylum-level trends were observed (Figure 22). In group 0, Firmicutes (63%; now Firmicutes) predominated, followed by Bacteroidetes (25%; Bacteroidetes), with smaller contributions from Actinobacteria (5%) and Proteobacteria (6%). In group 1, Firmicutes decreased to 50% while Bacteroidetes increased to 42%, and this pattern persisted in group 2 (Firmicutes 48%; Bacteroidetes 45%). Actinobacteria and Proteobacteria declined across groups, indicating a progressive shift in dominant community structure with increasing meditation engagement.

5.2.4 Taxonomical differences

At the genus level, 103 taxa exceeded 0.1% relative abundance. The most abundant genera included *Bacteroides* (18.4%), *Alistipes* (7.0%), *Faecalibacterium* (4.5%), *Blautia* (2.4%), *Ruminococcaceae* UCG-002 (2.4%), and *Roseburia* (2.2%), with *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus*, and *Collinsella* representing the leading genera overall. The heatmap in Figure 23 summarizes the relative abundance of the top 20 genera across low, medium, and high meditation groups. *Bacteroides* was dominant across all groups (26.98% in medium to 34.39% in high), followed by *Faecalibacterium*, *Prevotella*, *Ruminococcus*, and *Collinsella*. Notably, *Prevotella* peaked in the medium group (17.01%), whereas *Bacteroides* and *Faecalibacterium* increased progressively from low to high

meditation. Several genera (e.g., *Sutterella*, *Collinsella*, *Ruminococcus*) were relatively higher in the medium group, while others such as *Streptococcus*, *Lachnospira*, and *Alistipes* remained comparatively stable. Collectively it suggests that specific bacterial taxa may vary with meditation depth, supporting a potential association between meditative practice intensity and gut microbiota composition.

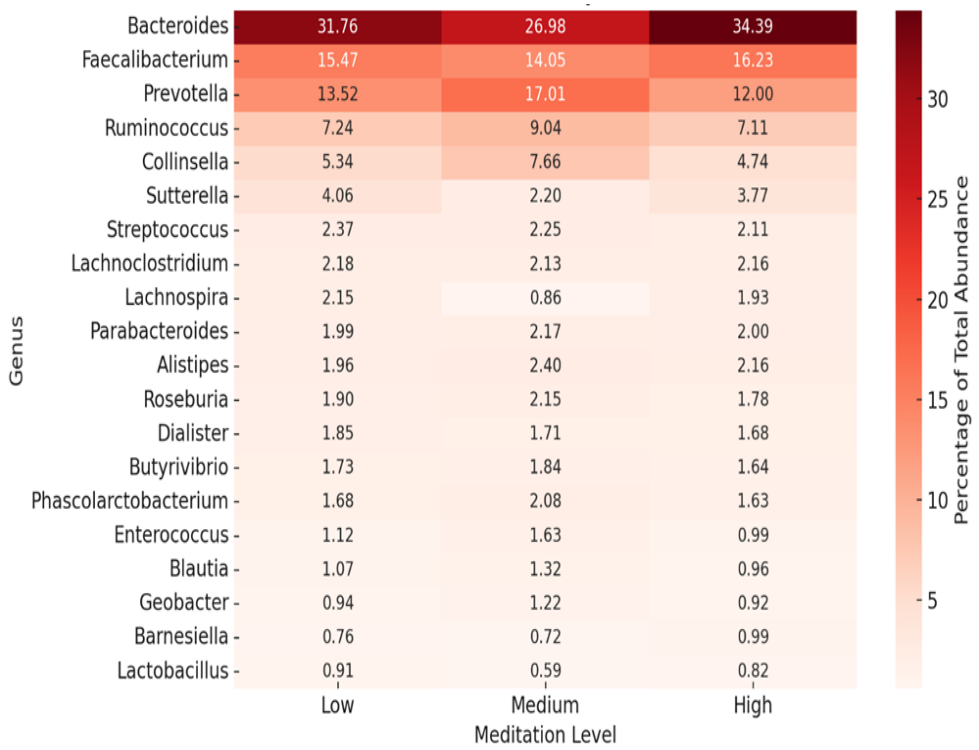


Figure 23. Relative contribution of bacterial genera to total community abundance across meditation (Ghaffar et al.,2026)

Comparison of the most abundant genera (relative abundance > 1%) between the Aikido and meditation groupings showed both shared and group-specific patterns (Figure 23). Ten genera exceeded the 1% threshold when samples were stratified by Aikido level, whereas 13 genera exceeded this threshold when stratified by meditation level. Seven genera were common to both groupings *Prevotella*, *Bacteroides*, *Roseburia*, *Enterococcus*, *Ruminococcus*, *Faecalibacterium*, and *Alistipes* all well-established constituents of the human gut microbiota. Examination of relative-abundance shifts across meditation-depth categories suggested greater variability in these shared genera within the meditation groups, and these differences were statistically significant ($p = 0.0001$), whereas fluctuations across Aikido practice levels were comparatively smaller. Three genera were detected only in the Aikido-based comparison (*Phascolarctobacterium*, *Oscillibacter*, *Dialister*), while six genera were unique to the meditation-based comparison (*Collinsella*, *Sutterella*, *Lachnospira*, *Parabacteroides*,

Butyrivibrio, *Lachnoclostridium*). Collectively, these findings indicate that distinct microbial signatures may be differentially associated with practice-related factors, with meditation depth showing stronger compositional shifts than Aikido frequency. Prevotella showed a slight increase relative to the lowest meditation-score group (Figure 24).

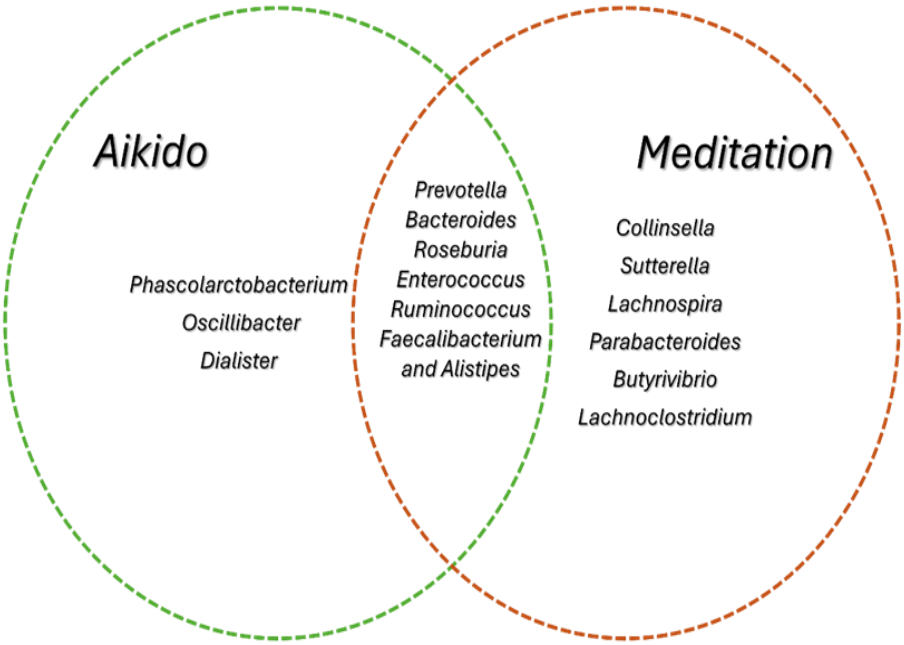


Figure 24. Venn diagram showing the distribution of bacterial genera (cutoff >1% relative abundance) between the Aikido and Meditation groups (Ghaffar et al.,2026)

The results obtained indicated significant shifts in gut microbiota composition among high-meditation practitioners, characterized by increased relative abundances of *Bacteroides* and *Faecalibacterium*, with a modest rise in *Prevotella*. With respect to Aikido intensity, the moderate-intensity group was associated with an enrichment of *Firmicutes*. Overall, these findings suggest that Aikido as a "meditation in movement" practice may support a more balanced *Bacteroidetes/Firmicutes* ratio, consistent with intestinal eubiosis and potentially contributing to gut health and well-being.

5.3 RESULTS OF REGULATION OF GUT MICROBIOTA IN TAICHI & AIKIDO ATHLETES

5.3.1 Participant Information and Study Design (General Characteristics of the Subjects)

A total of 42 fecal samples were collected from adult practitioners of two meditative martial arts Tai Chi and Aikido (Table 11) both of which combine structured physical movement with meditative elements. As described in the Methods (Section 4.3.1), the Aikido cohort comprised the same participants characterised in Aikido study; the Tai Chi cohort was recruited independently as part of this next study . Both datasets were analysed within a unified PRIMER 7 workspace using identical normalisation and multivariate settings, minimising any potential batch effects. All diversity analyses were performed at the genus taxonomic level, with phylum-level analyses conducted in parallel to characterise broad community structure.

Descriptive analyses were conducted for anthropometric, sociodemographic, and lifestyle characteristics. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). For analytical purposes, participants were stratified according to two independent factors: (i) meditative movement practice (Tai Chi vs. Aikido) and (ii) sex (male vs. female). The main effects of practice type and meditation depth were evaluated, and additional sex-stratified analyses were performed within each practice group. The term “exercise + gender” refers solely to these sex-stratified comparisons and does not indicate a distinct or combined experimental group. Data were collected on age, sex, BMI, educational attainment, smoking and alcohol habits, dietary patterns, physical activity level, meditation score, and practice intensity/level. Overall, the two practice groups were comparable across most variables; however, significant differences were observed in sex distribution and MEDI score (Table 11). Physical activity was quantified using metabolic equivalent of task (MET) units. Data are presented as mean ± standard deviation (SD). No significant differences were detected for the remaining variables based on independent-samples t-test comparisons.

Table 11. Socio-demographic characteristics of Tai Chi and Aikido participants.

Variable	Value Aikido	Value Taichi	P-value
Gender			
female	9 (39.13 %)	14 (73.68 %)	0.03
male	14 (60.87 %)	5 (26.31 %)	
Age			
18 – 30	1 (4.17 %)	1 (5.26 %)	
31 – 65	20 (83.33 %)	12 (63.16 %)	0.29

> 65	3 (12.5 %)	6 (31.58 %)	
Educational level			
Middle school graduation	1(4.17 %)	0(0.00 %)	
High school degree	9(37.50 %)	6(40.00 %)	
Bachelor's degree	1(4.17 %)	2(13.33 %)	
Master's degree	9(37.50 %)	7(46.67 %)	0.36
PhD	4(16.67 %)	0(0.00 %)	
Smoking			
No	16(66.67 %)	14(73.68 %)	0.74
Yes	8(33.33 %)	5(26.32%)	
Smoking frequency			
At least once/day	6(75.00 %)	2(40.00 %)	
4-6 times/week	1(12.50 %)	2(40.00 %)	0.42
Maximum 3 times/week	1(12.50 %)	1(20.00 %)	
BMI			
Normal weight	17(70.83 %)	12(63.16 %)	
Underweight	0(0.00 %)	1(5.26 %)	
Overweight	6(25.00 %)	6(31.58 %)	0.50
Obese	1(4.17 %)	0(0.00 %)	
Diet			
No specific diet	10(41.67 %)	5(26.32 %)	
Mediterranean	8(33.33 %)	12(63.16 %)	
Vegetarian	3(12.50 %)	1(5.26 %)	
Requested by physician	1(4.17 %)	0(0.00 %)	
Caloric restriction	1(4.17 %)	0(0.00 %)	0.3
Gluten free	0(0.00 %)	1(5.26 %)	
Other	1(4.17 %)	0(0.00 %)	
Alcohol consumption			
No alcohol	0(0.00 %)	3(15.79 %)	
Often (> 5 times/month)	11(45.83 %)	6(31.58 %)	
Sometimes (3-4 times/month)	10(41.67 %)	7(36.84 %)	0.20
Occasionally (1-2 times/month)	3(12.50 %)	3(15.79 %)	
Aikido practice			
< 300 min/week	14(58.33 %)	9(47.37 %)	0.50
> 300 min/week	10(41.67 %)	10(52.63 %)	
General physical activity			
Partially active	4(16.67 %)	6(31.58 %)	
Physically active	20(83.33 %)	13(68.42 %)	0.29
MEDI score			
Low (0 – 60)	10(41.67 %)	17(89.47 %)	
Medium (60 – 80)	7(29.17 %)	1(5.26 %)	0.005
High (80 – 120)	7(29.17 %)	1(5.26 %)	
Meditation time			
< 60 min/week	13(54.17 %)	6(31.58 %)	
> 60 min/week	11(45.83 %)	11(57.89 %)	0.13
Not available	0(0.00 %)	2(10.53 %)	

5.3.2 Alpha diversity indices

Alpha diversity was quantified with four complementary metrics computed in PRIMER 7 using the DIVERSE routine: species richness (S), Margalef index, Shannon's index (H′), and Simpson's index ($1 - \lambda'$). Differences between groups were assessed using the Mann–Whitney U test or Kruskal–Wallis test as appropriate, with effect sizes reported as rank-biserial r and eta-squared (η^2), respectively. Multiple testing was controlled using the Benjamini–Hochberg (BH) false discovery rate (FDR) procedure across 20 comparisons. Because the sequence data were summarised at the genus level, species richness (S) reflects the number of distinct genera

per sample rather than individual species.

Aikido practitioners exhibited substantially higher alpha diversity than Tai Chi practitioners across all four metrics (Figure 25). Species richness was significantly greater in Aikido (median S = 157) than in Tai Chi (median S = 105; U = 7.0, r = −0.82, FDR adj p < 0.001), indicating that Aikido participants harboured a markedly richer gut microbiota. Margalef index confirmed this pattern (Aikido median d = 14.69 vs. Tai Chi median d = 9.87; U = 6.0, r = −0.83, FDR adj p < 0.001). Shannon's index was similarly elevated in Aikido (median H′ = 2.39) relative to Tai Chi (median H′ = 1.98; U = 60.0, r = −0.52, FDR adj p = 0.010), reflecting greater combined richness and evenness. Simpson's index showed the same direction but a smaller effect (Aikido median $1 - \lambda' = 0.813$ vs. Tai Chi 0.739; U = 71.0, r = −0.44, FDR adj p = 0.041). Taken together, these findings indicate that regular Aikido practice is associated with a significantly more diverse gut microbiota compared with Tai Chi.

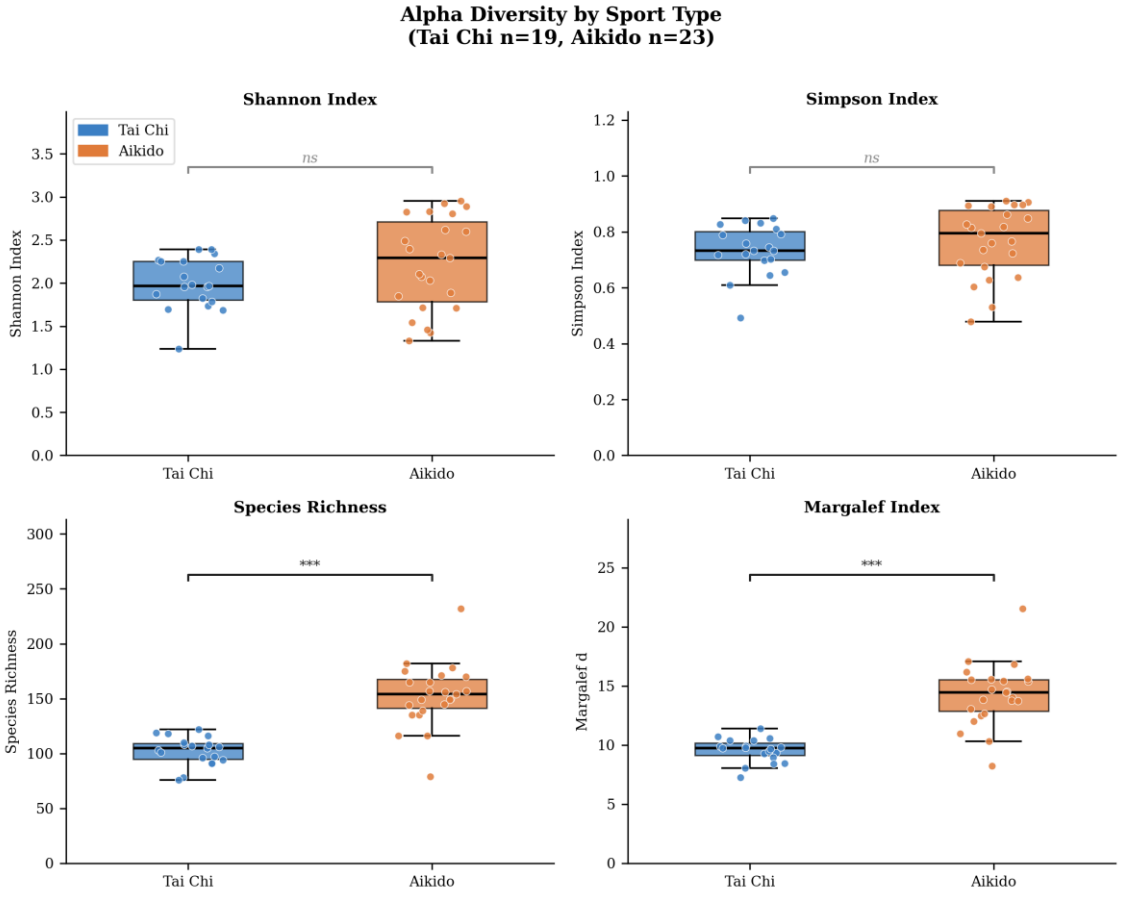


Figure 25. Alpha diversity by sport type. Box-and-whisker plots comparing species richness (S), Margalef index, Shannon index, and Simpson index between Tai Chi (n = 19, blue) and Aikido (n = 23, orange) practitioners. Significance brackets show Mann–Whitney U results after Benjamini–Hochberg FDR correction: *** FDR adj p < 0.001, ** FDR adj p < 0.01, * FDR adj p < 0.05.

Participants were grouped by self-reported meditation practice: no regular practice (level 0, n

= 24), occasional practice (level 1, n = 8), and regular or intensive practice (level 2, n = 10). Kruskal–Wallis tests revealed no statistically significant differences in any alpha diversity metric across meditation levels after FDR correction (Figure 26). Species richness did not differ by meditation group (H = 3.85, η^2 = 0.07, FDR adj p = 0.271). Similarly, Margalef index (H = 3.22, η^2 = 0.05, FDR adj p = 0.340), Shannon index (H = 1.01, η^2 = 0.00, FDR adj p = 0.732), and Simpson index (H = 0.98, η^2 = 0.00, FDR adj p = 0.732) were all non-significant.

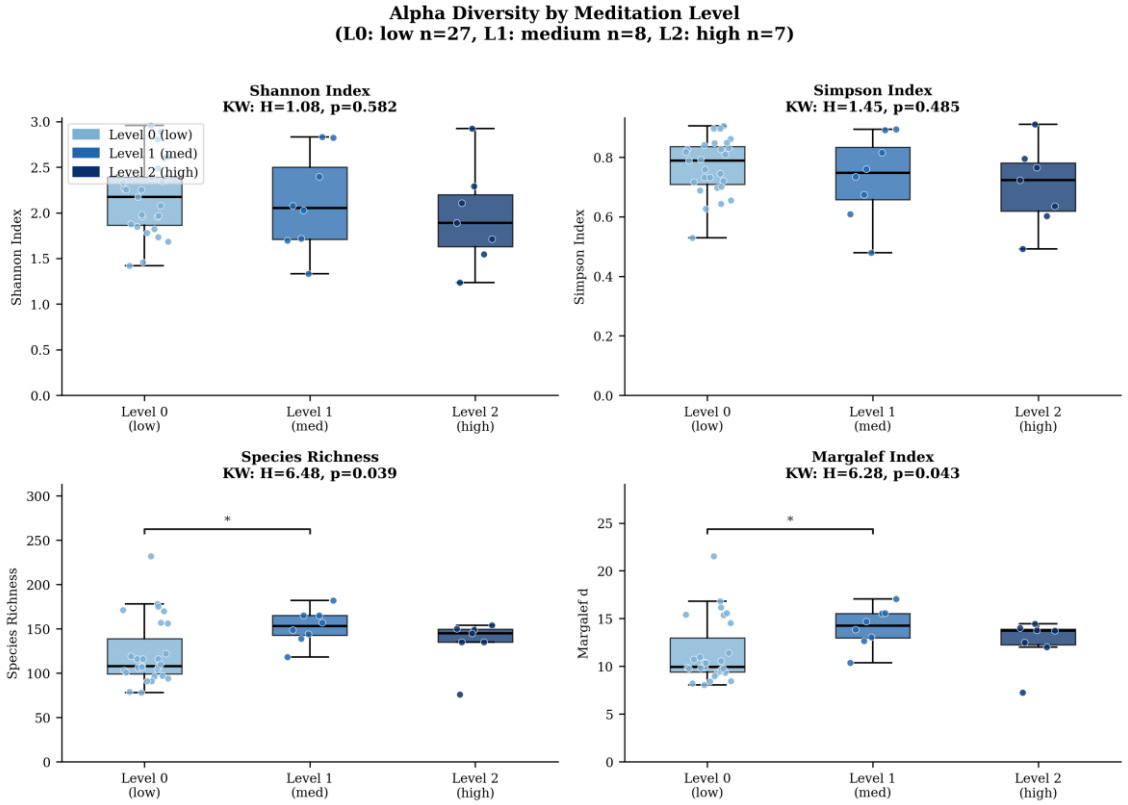


Figure 26. Alpha diversity by meditation level. Box plots of four alpha diversity indices stratified by self-reported meditation practice level: no practice (level 0, n = 24), occasional practice (level 1, n = 8), and regular or intensive practice (level 2, n = 10). Kruskal–Wallis p-values after Benjamini–Hochberg FDR correction are shown; no significant differences were detected (all FDR adj p > 0.05).

Alpha diversity was compared between male (n = 24) and female (n = 18) participants (Figure 27). No significant differences were detected for any of the four metrics following FDR correction. Species richness was similar between males (median S = 154) and females (median S = 145; U = 204.0, r = 0.06, FDR adj p = 0.870). Margalef index (U = 208.0, r = 0.08, FDR adj p = 0.870), Shannon index (U = 216.5, r = 0.13, FDR adj p = 0.870), and Simpson index (U = 222.0, r = 0.16, FDR adj p = 0.870) were likewise non-significant.

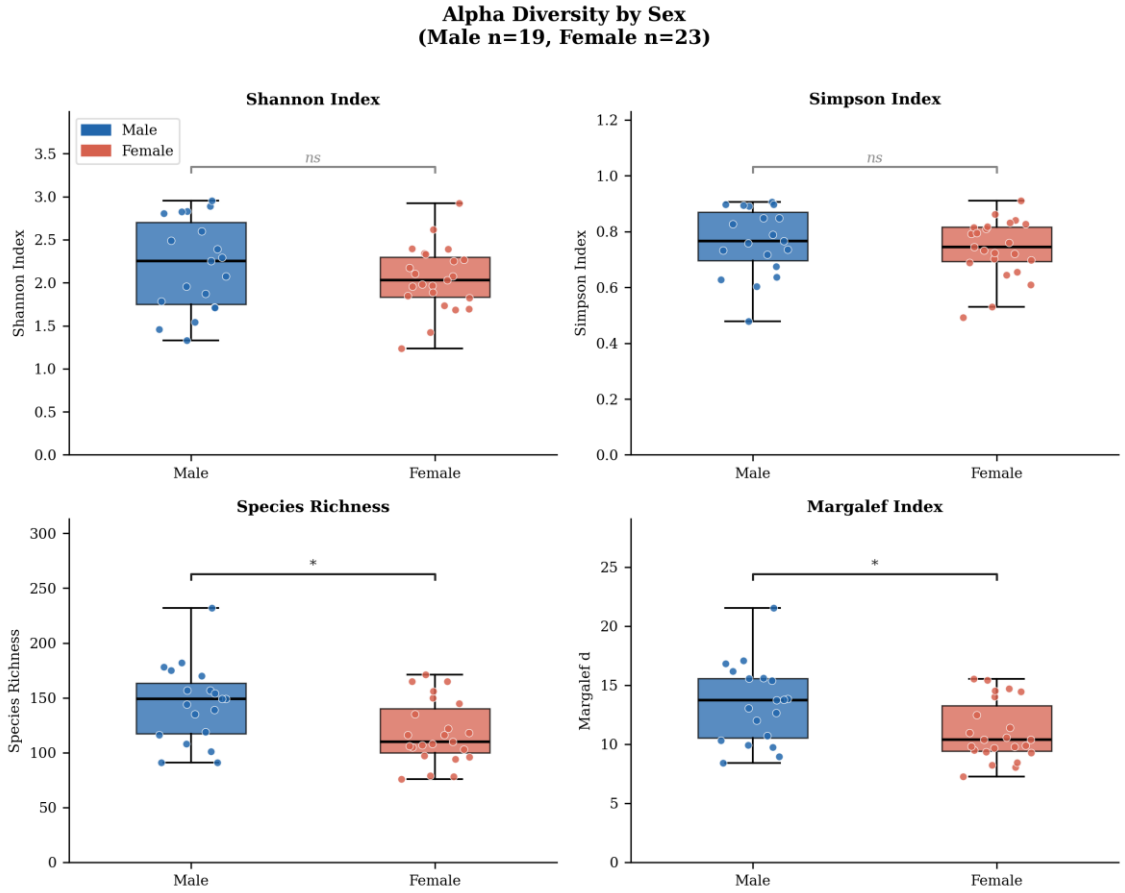


Figure 27. Alpha diversity by sex. Box plots comparing species richness (S), Margalef index, Shannon index, and Simpson 1 − λ′ between male (n = 24, blue) and female (n = 18, pink) participants. Mann–Whitney U test p-values after Benjamini–Hochberg FDR correction are displayed; no significant differences were observed (all FDR adj p > 0.05).

Participants were categorised by BMI as normal weight (BMI < 25 kg/m², n = 19), overweight (25 ≤ BMI < 30 kg/m², n = 17), or obese (BMI ≥ 30 kg/m², n = 6). Kruskal–Wallis tests indicated no significant variation in alpha diversity across BMI categories after FDR correction (Figure 28). Species richness did not differ ($H = 4.23$, $\eta^2 = 0.08$, FDR adj $p = 0.271$), nor did Margalef index ($H = 4.09$, $\eta^2 = 0.07$, FDR adj $p = 0.271$), Shannon index ($H = 0.79$, $\eta^2 = 0.00$, FDR adj $p = 0.732$), or Simpson index ($H = 0.55$, $\eta^2 = 0.00$, FDR adj $p = 0.732$).

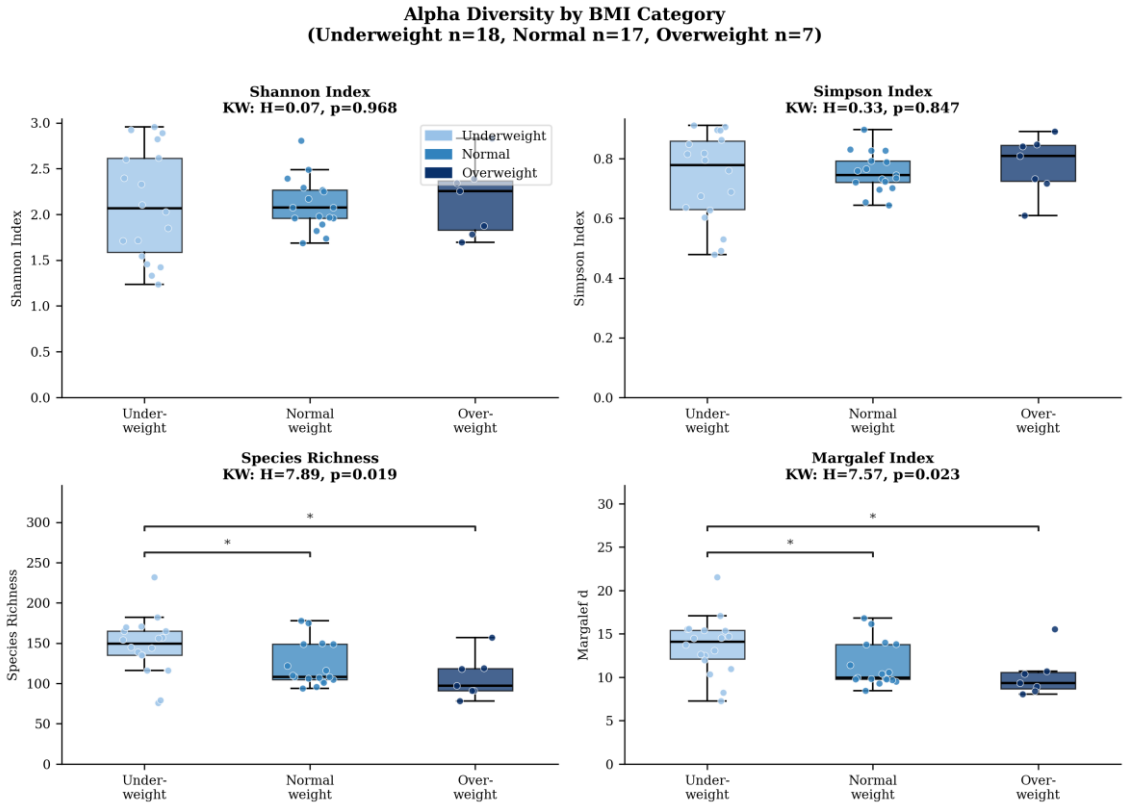


Figure 28. Alpha diversity by BMI category. Box plots of four alpha diversity indices stratified by BMI category: normal weight (BMI < 25, n = 19, green), overweight (25 ≤ BMI < 30, n = 17, amber), and obese (BMI ≥ 30, n = 6, red). Kruskal–Wallis p-values after Benjamini–Hochberg FDR correction are shown; no significant group differences were detected (all FDR adj p > 0.05).

Physical activity (PA) was categorised as low (level 0, n = 23) or moderate-to-high (level 1, n = 19). No significant differences in alpha diversity were found between PA groups after FDR correction (Figure 29). Species richness (U = 195.0, r = 0.01, FDR adj p = 0.870), Shannon index (U = 210.5, r = 0.10, FDR adj p = 0.870), Simpson 1 − λ′ (U = 201.5, r = 0.04, FDR adj p = 0.870), and Margalef index (U = 200.0, r = 0.03, FDR adj p = 0.870) were all non-significant. These findings indicate that self-reported physical activity level does not independently predict within-sample gut microbiota diversity after controlling for multiple comparisons.

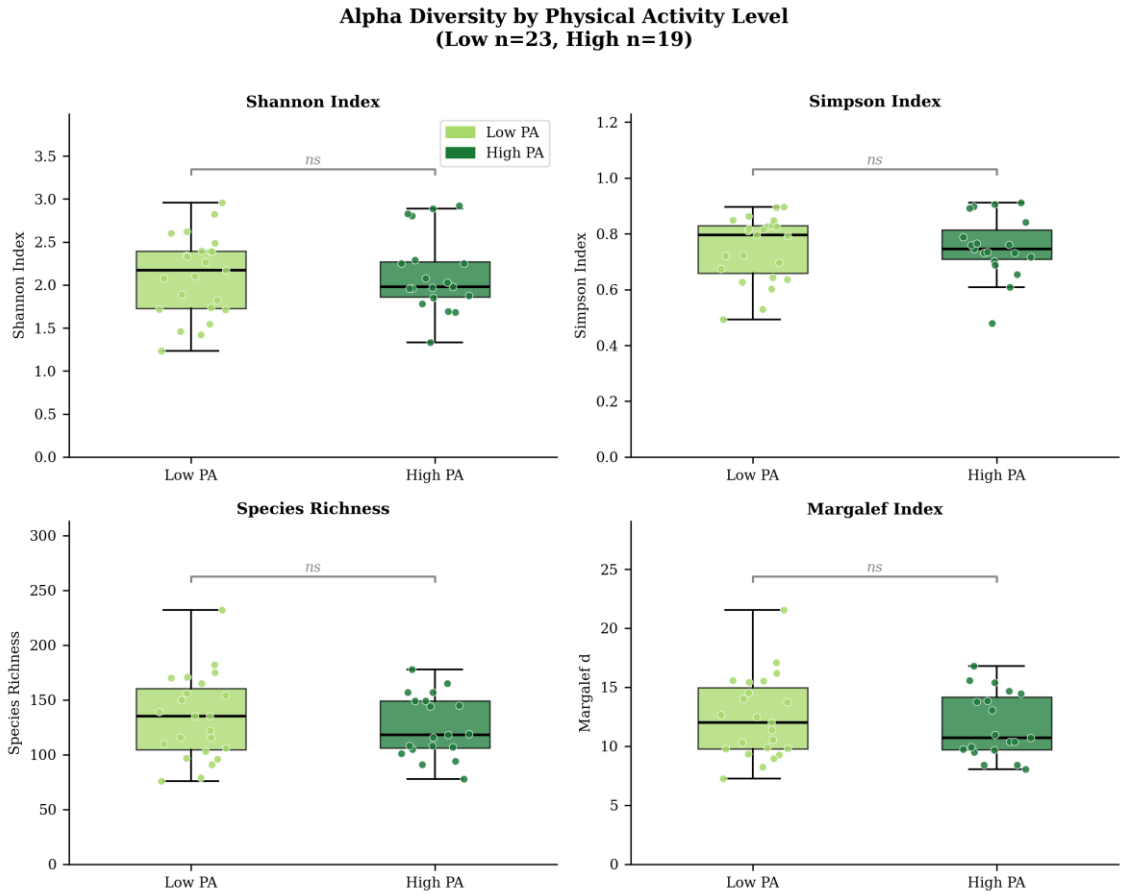


Figure 29. Alpha diversity by physical activity level. Box plots comparing species richness (S), Margalef index, Shannon index, and Simpson 1 − λ′ between low physical activity (level 0, n = 23, grey) and moderate-to-high physical activity (level 1, n = 19, teal) groups. Mann–Whitney U test p-values after Benjamini–Hochberg FDR correction are shown; no significant differences were detected (all FDR adj p > 0.05).

The figure 30 provides a consolidated overview of all four alpha diversity metrics across all five grouping factors simultaneously. Sport type produced the largest and most consistent differences, with Aikido practitioners demonstrating significantly higher diversity on all metrics. Meditation level, sex, BMI, and physical activity level did not produce significant differences in alpha diversity after FDR correction, confirming that the primary driver of within-sample microbiota diversity in this cohort is the martial arts discipline rather than demographic or lifestyle factors.

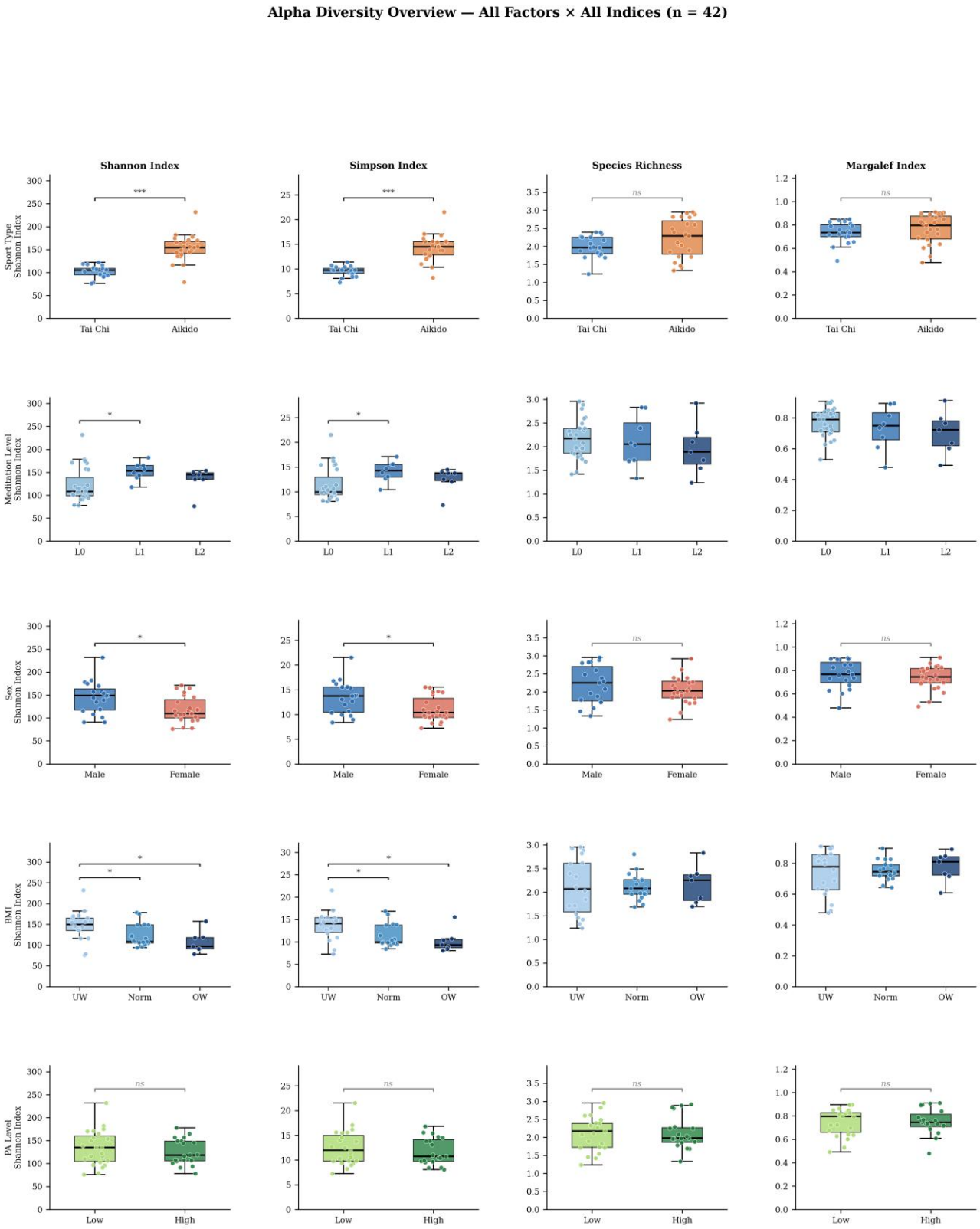


Figure 30. Combined overview of alpha diversity across all grouping factors. Consolidated panel displaying species richness (S), Margalef index, Shannon index, and Simpson index for each of the five grouping factors: sport type, meditation level, sex, BMI category, and physical activity level. Significance annotations reflect Benjamini–Hochberg FDR-corrected Mann–Whitney U or Kruskal–Wallis test results. Sport type was the only factor with statistically significant effects on alpha diversity.

5.3.3 Beta Diversity and Compositional Differences (PCoA)

Beta diversity was evaluated using Bray–Curtis dissimilarity and visualised by principal coordinates analysis (PCoA). The first two PCoA axes explained 33.96% and 12.89% of the total variation at genus level, respectively. Visual inspection suggested partial separation between Aikido and Tai Chi participants, consistent with differences in overall community composition (Figure 31). Group-level dispersion was illustrated using 95% confidence ellipses.

To formally test for group differences in community composition, both Analysis of Similarities (ANOSIM) and Permutational Multivariate Analysis of Variance (PERMANOVA) were performed on the Bray–Curtis distance matrix, each with 9,999 permutations. ANOSIM was executed in PRIMER 7; PERMANOVA was implemented using a custom Python function based on the Anderson (2001) formulation with a fixed random seed (42) for reproducibility. PERMANOVA partitions total multivariate variance into between-group (SSB) and within-group (SSW) components and reports an F-statistic, an R^2 value (proportion of variance explained), and a permutation-based p-value.

Sport type was the only factor to reach statistical significance in the PERMANOVA analysis. Sport type explained 15.7% of the total variance in Bray–Curtis dissimilarity ($F = 7.441$, $df = 1,40$, $R^2 = 0.157$, $p = 0.0001$), and ANOSIM confirmed a significant difference in community structure between Tai Chi and Aikido ($R = 0.462$, $p = 0.0001$). No other single factor reached significance: meditation level (PERMANOVA: $F = 0.680$, $df = 2,39$, $R^2 = 0.033$, $p = 0.760$; ANOSIM: $R = 0.033$, $p = 0.201$), sex (PERMANOVA: $F = 0.699$, $df = 1,40$, $R^2 = 0.017$, $p = 0.601$; ANOSIM: $R = 0.021$, $p = 0.226$), BMI (PERMANOVA: $F = 0.526$, $df = 2,39$, $R^2 = 0.026$, $p = 0.845$), and physical activity level (PERMANOVA: $F = 0.485$, $df = 1,40$, $R^2 = 0.012$, $p = 0.872$) were all non-significant. However, when sport type and meditation level were considered jointly, the combined Sport × Meditation grouping explained a substantially larger proportion of variance (PERMANOVA: $F = 2.610$, $df = 5,36$, $R^2 = 0.266$, $p = 0.0001$), confirming a meaningful interaction between discipline and meditative practice in shaping gut microbiota composition. ANOSIM further identified significant differences across meditation categories ($R = 0.191–0.240$, $p < 0.05$) and a modest Sport × Sex interaction ($R = 0.091$, $p = 0.049$). Given the very small sample size in some meditation-level strata, interaction effects should be interpreted cautiously and regarded as hypothesis-generating. Collectively, these findings support a potential role for meditative practice characteristics in shaping gut microbiota composition within this physically active cohort.

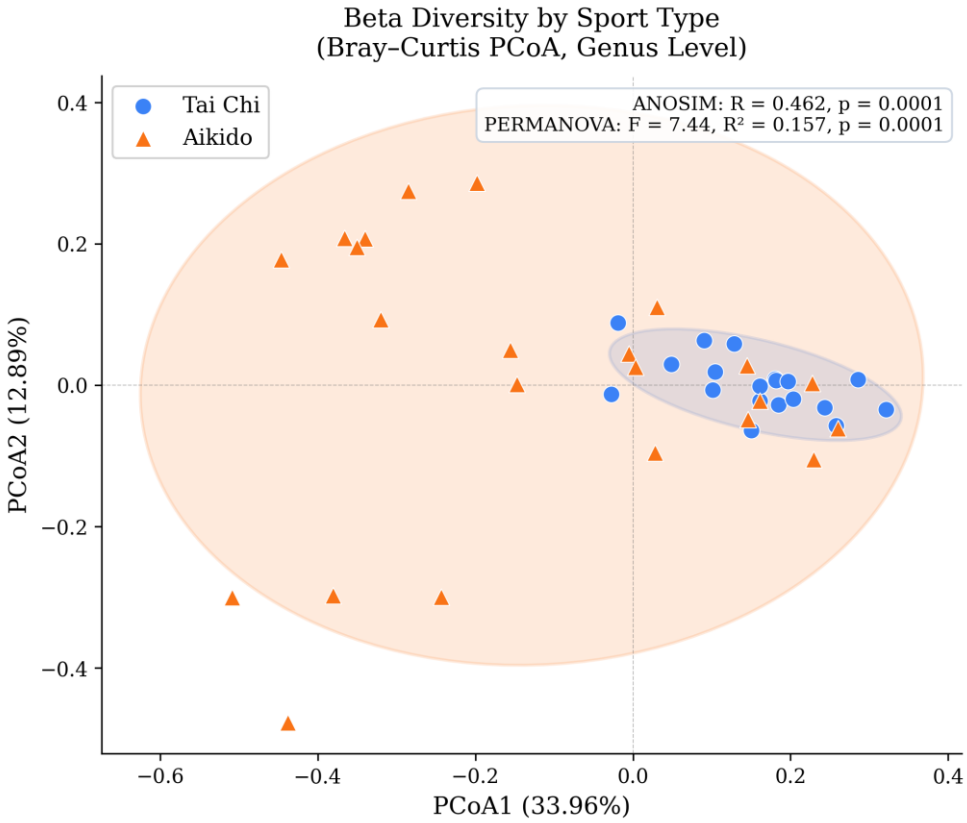


Figure 31. Beta diversity by sport type. Principal Coordinates Analysis (PCoA) of Bray–Curtis dissimilarities at genus level coloured by martial arts discipline: Tai Chi (blue circles, n = 19) and Aikido (orange triangles, n = 23). Each point represents one participant; 95% confidence ellipses are drawn for each group. PCoA1 and PCoA2 explain 33.96% and 12.89% of total variance, respectively. ANOSIM (R = 0.462, p = 0.0001) and PERMANOVA (F = 7.44, R^2 = 0.157, p = 0.0001) results are annotated on the figure.

At the genus level (Figure 32a), the first two PCoA axes explained 33.96% and 12.89% of the total variance, respectively. Aikido and Tai Chi samples exhibited partial separation, although substantial overlap remained, indicating only moderate between-group differences in genus-level community composition. The taxa most strongly associated with this separation included Prevotella (relatively more abundant in Tai Chi) and Bacteroides (relatively more abundant in Aikido), with additional contributions from Sutterella, Ruminococcus, Collinsella, Dialister, and Lachnospira. Overall, these findings suggest that a limited set of genera contributes disproportionately to the observed compositional variation between practices. At the phylum level (Figure 32b), PCoA1 and PCoA2 accounted for 69.15% and 13.32% of the variance, respectively, and group separation was more pronounced than that observed at the genus level. This pattern was primarily driven by differences in the relative abundance of Firmicutes (higher in Tai Chi) and Bacteroidetes (higher in Aikido), with smaller contributions from Proteobacteria, Actinobacteria, and Cyanobacteria. Taken together, these analyses indicate that Aikido and Tai Chi practitioners differ in gut microbiota composition at both taxonomic resolutions, with the clearest distinction emerging at the phylum level and largely reflecting

shifts in the Firmicutes/Bacteroidetes balance.

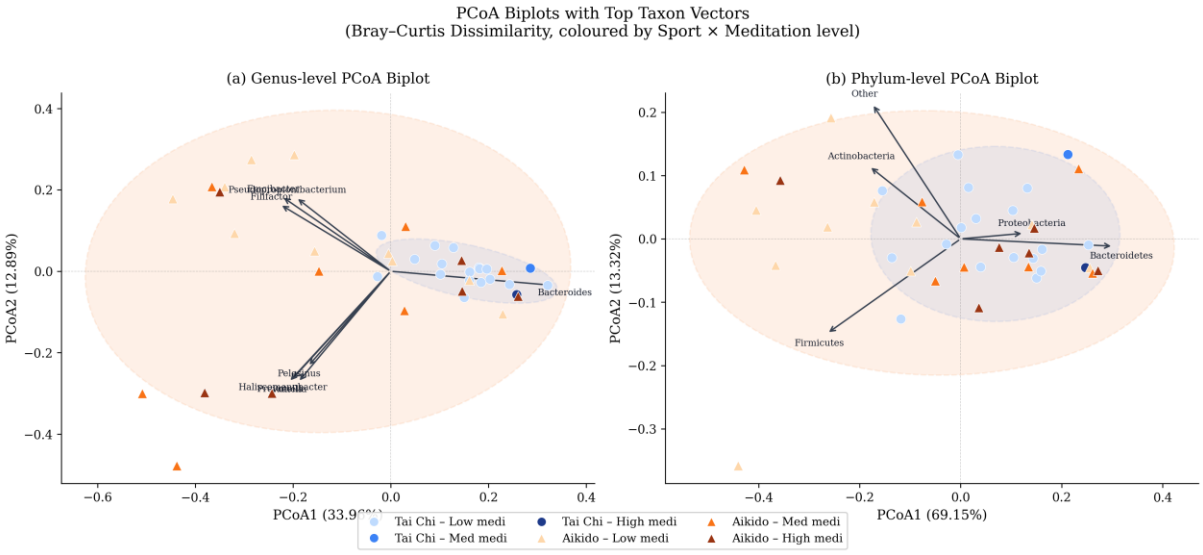


Figure 32. PCoA biplots at genus and phylum levels coloured by sport type and meditation. (a) Genus-level PCoA biplot (Bray–Curtis, PCoA1 = 33.96%, PCoA2 = 12.89%) with samples coloured by sport type × meditation level: Tai Chi in shades of blue and Aikido in shades of orange, with colour intensity increasing with meditation depth (light = none, medium = occasional, dark = regular). Dashed ellipses indicate 95% confidence regions per sport group. Genus vectors show the top taxa most strongly correlated with the ordination axes. (b) Phylum-level PCoA biplot (PCoA1 = 69.15%, PCoA2 = 13.32%) with phylum vectors highlighting the dominant contributions of Firmicutes and Bacteroidetes to compositional variation.

PCoA ordinations stratified by meditation level within the Aikido group (Figure 33) revealed structured patterns of variation, with meditation strata forming partially distinct yet overlapping clusters. Within the Aikido group, a gradual shift in community composition across meditation levels was observed, with modest separation along the first two PCoA axes (28.9% and 13.6% of explained variance, respectively) and some overlap between levels. Genera contributing most strongly to this gradient included Bacteroides, Parabacteroides, Flavonifractor, and Pelosinus, suggesting that increasing meditation depth may be associated with compositional changes in Aikido practitioners.

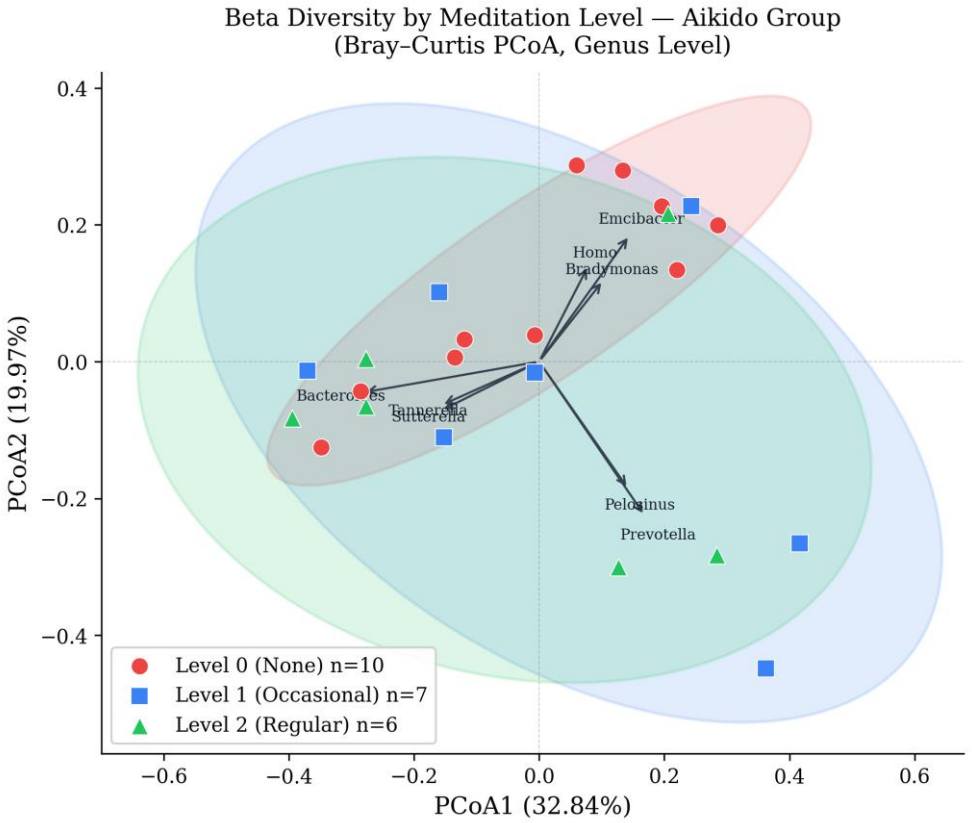


Figure 33. Beta diversity by meditation level within the Aikido group. Within-group PCoA of Bray–Curtis dissimilarities computed on Aikido participants only (n = 23), coloured by meditation level: no practice (red circles), occasional practice (blue squares), and regular practice (green triangles). Shaded ellipses show 95% confidence regions for groups with n ≥ 3. Genus vectors indicate the top taxa most strongly correlated with the within-group ordination axes.

Within the Tai Chi group (Figure 34), samples also showed partial segregation by meditation level. Participants at meditation level 0 (non-meditators) clustered more tightly, whereas samples from higher meditation levels were more dispersed across the ordination space, again along axes explaining 28.9% and 13.6% of the variance. Taxa such as Sporosarcina, Odoribacter, and Bacteroides contributed prominently to the observed separation, indicating specific genus-level shifts associated with meditation level in Tai Chi practitioners. These visual patterns were supported by ANOSIM results, which indicated a significant effect of meditation level on beta diversity across the cohort ($R = 0.191$, $p = 0.035$). Moreover, a strong Sport × Meditation interaction was detected ($R = 0.296$, $p = 0.001$), suggesting that the association between meditation depth and gut microbiota composition may differ between Aikido and Tai Chi.

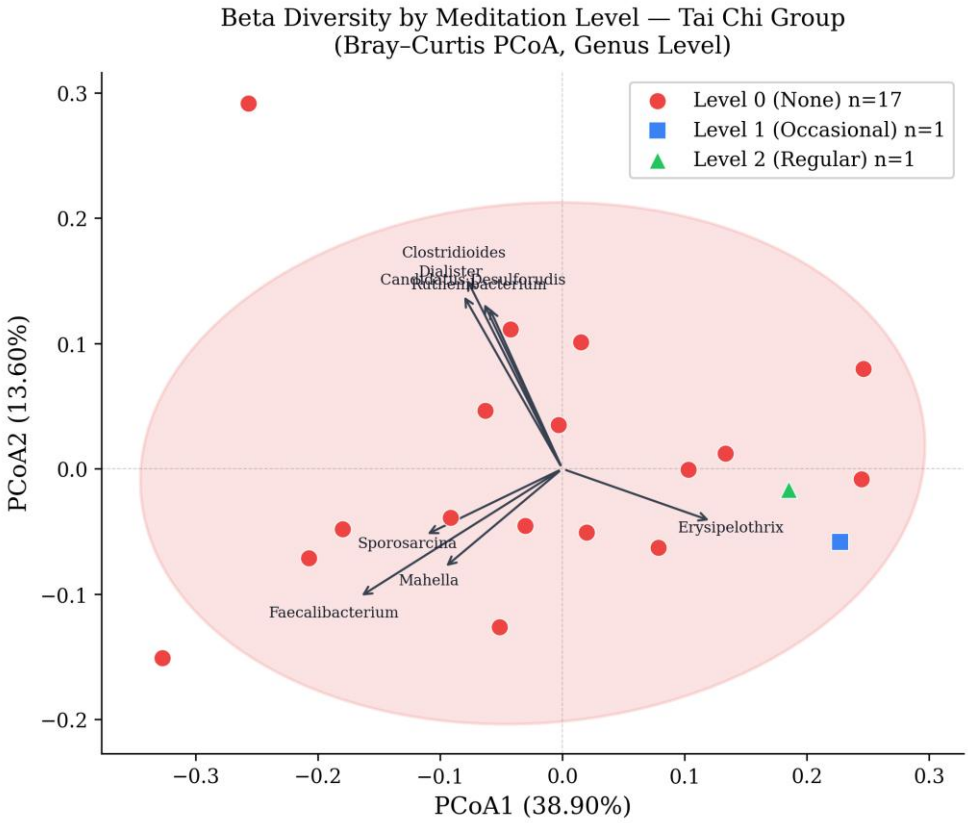


Figure 34. Beta diversity by meditation level within the Tai Chi group. Within-group PCoA of Bray–Curtis dissimilarities computed on Tai Chi participants only (n = 19), coloured by meditation level: no practice (red circles), occasional practice (blue squares), and regular practice (green triangles). Shaded ellipses show 95% confidence regions for groups with n ≥ 3. Genus vectors indicate the top taxa most strongly correlated with the within-group ordination axes.

When Tai Chi and Aikido participants were considered jointly across meditation levels (Figure 35a), the ordination patterns indicated that a limited set of genera most notably Prevotella, Bacteroides, Ruminococcus, and Collinsella contributed most strongly to the observed compositional variation. Prevotella was associated with higher values along PCoA1, whereas Bacteroides was associated with lower PCoA1 values, consistent with opposing gradients in community structure. At the genus level, PCoA1 and PCoA2 explained 67.54% and 10.75% of the total variance, respectively. Although the dispersion of confidence ellipses suggested that meditation strata may be associated with shifts in microbiota structure, separation was incomplete, indicating substantial overlap between groups. Spearman correlation analysis between the continuous meditation score and the relative abundance of key genera did not identify any significant associations (all $p > 0.05$). Sex-stratified PCoA biplots pooling both practices (Figure 35b) suggested only modest differences between male and female participants, with partial clustering by sex particularly along PCoA1 yet with extensive overlap between the 95% confidence ellipses. As observed in the meditation-stratified ordinations, Prevotella and Bacteroides were the principal drivers of variation along PCoA1, while

Ruminococcus, Collinsella, and Dialister contributed more modestly to sample dispersion. Collectively, these results suggest that sex may play a secondary role in shaping community composition in this cohort, with comparatively stronger structuring linked to practice-related factors and meditation-related patterns.

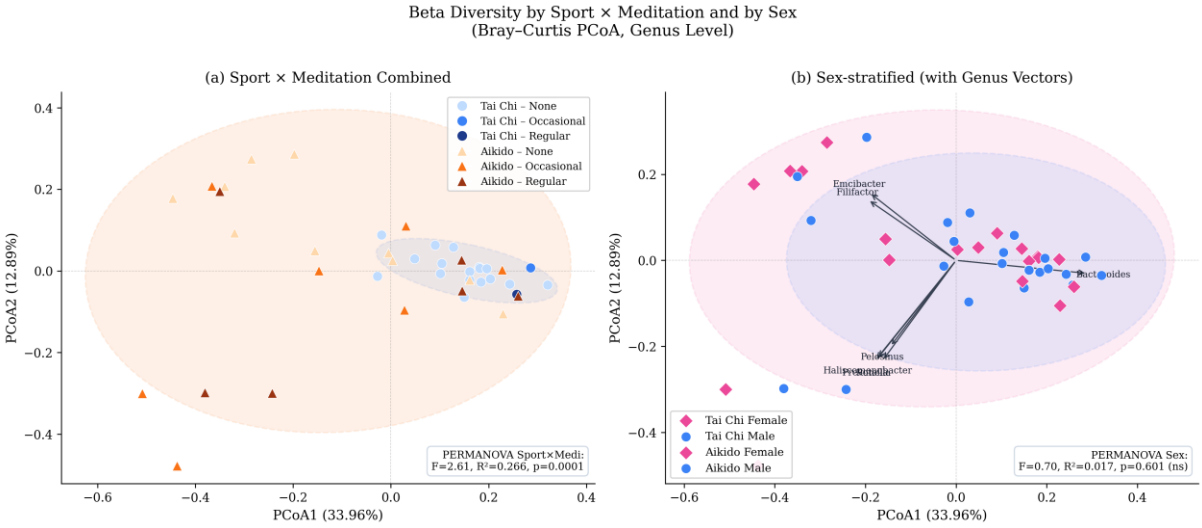


Figure 35. Beta diversity by sport × meditation and by sex. (a) PCoA ordination (PCoA1 = 33.96%, PCoA2 = 12.89%) with samples coloured by the combined sport type and meditation level: Tai Chi in shades of blue (light to dark = increasing meditation) and Aikido in shades of orange. Dashed ellipses indicate 95% confidence regions per sport group. PERMANOVA for the Sport × Meditation combined grouping: $F = 2.61$, $R^2 = 0.266$, $p = 0.0001$. (b) The same ordination with samples coloured by sex and sport type, with genus biplot vectors overlaid. PERMANOVA for sex: $F = 0.70$, $R^2 = 0.017$, $p = 0.601$ (non-significant).

5.3.4 Genus & Phylum Level Microbiota Composition and Differences

A heatmap of genus-level mean relative abundance, normalized by Z-scores, was generated to compare gut microbiota composition between Aikido and Tai Chi practitioners. Of the 503 genera detected across all samples, the 50 most abundant genera were visualized (Figure 36). The heatmap encompassed a wide range of taxa, including prominent genera such as *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus*, *Alistipes*, *Sutterella*, *Collinsella*, *Roseburia*, *Blautia*, and *Lactobacillus*. Distinct abundance patterns were evident between the two practice groups, with several genera showing relatively higher abundance in one group compared with the other, as reflected by positive versus negative Z-scores. Z-score normalization enabled comparison of relative differences independent of absolute abundance. In the heatmap, genera are displayed along the vertical axis and practice groups along the horizontal axis, with colour intensity representing standardized abundance values. Red hues (positive Z-scores) indicate genera with higher-than-average abundance in each group, whereas blue hues (negative Z-scores) denote lower-than-average abundance; a Z-score of zero corresponds to the mean abundance across both groups. Overall, genera such as *Bacteroides*,

Faecalibacterium, *Prevotella*, *Ruminococcus*, and *Alistipes* tended to be enriched in one practice group, whereas *Lactobacillus*, *Barnesiella*, and *Collinsella* were relatively more abundant in the other. To formally test genus-level differences between Aikido and Tai Chi practitioners, non-parametric Mann–Whitney U tests were applied to normalized relative abundances for each genus, followed by adjustment for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR). Of the 503 genera evaluated, five showed statistically significant differences between groups (FDR < 0.05): *Thioalkalivibrio*, *Pediococcus*, *Faecalibaculum*, *Butyrivibrio*, and *Rhodanobacter*. Notably, *Thioalkalivibrio*, *Pediococcus*, and *Faecalibaculum* exhibited particularly strong group differences, with FDR-adjusted p-values < 1.0×10^{-6}.

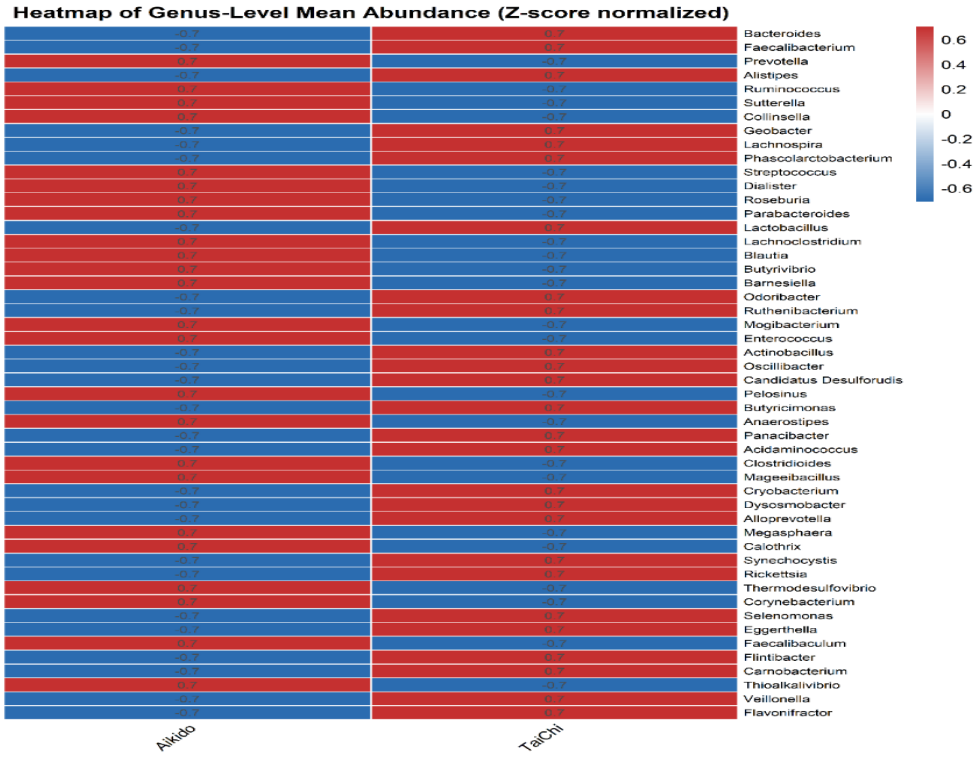


Figure 36. Genus-level abundance heatmap. Heatmap of normalized mean abundance (Z-scores) across 503 bacterial genera in Tai Chi and Aikido participants. Differences included higher Faecalibacterium and Roseburia in Aikido and greater Collinsella in Tai Chi (Ghaffar et al.,2026)

In addition, a heatmap of phylum-level mean relative abundance, standardized using Z-scores, was generated to compare gut microbiota composition between Aikido and Tai Chi practitioners (Figure 37). A total of 30 phyla were detected. Z-score normalization facilitated the visualization of between-group patterns across taxa with markedly different absolute abundances. Overall, the heatmap highlighted compositional differences between the two practice groups. Although *Firmicutes* and *Bacteroidetes* dominated both profiles, Aikido

samples exhibited stronger standardized signals for *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*, whereas Tai Chi samples showed a comparatively lower and more uniform phylum-level profile. Most low-abundance phyla displayed minimal or near-absent signals in both groups, consistent with limited representation across the cohort. Phylum-level differences between groups were formally evaluated using non-parametric testing with Benjamini Hochberg FDR correction. Five phyla differed significantly between Aikido and Tai Chi practitioners (FDR < 0.05): *Armatimonadetes*, *Candidatus Gracilibacteria*, *Planctomycetes*, *Nitrospirae*, and *Fusobacteria*. Among these, *Armatimonadetes* and *Candidatus Gracilibacteria* showed particularly strong evidence of between-group divergence (FDR-adjusted $p < 1.0 \times 10^{-4}$), suggesting distinct phylum-level ecological signatures associated with the two movement practices.

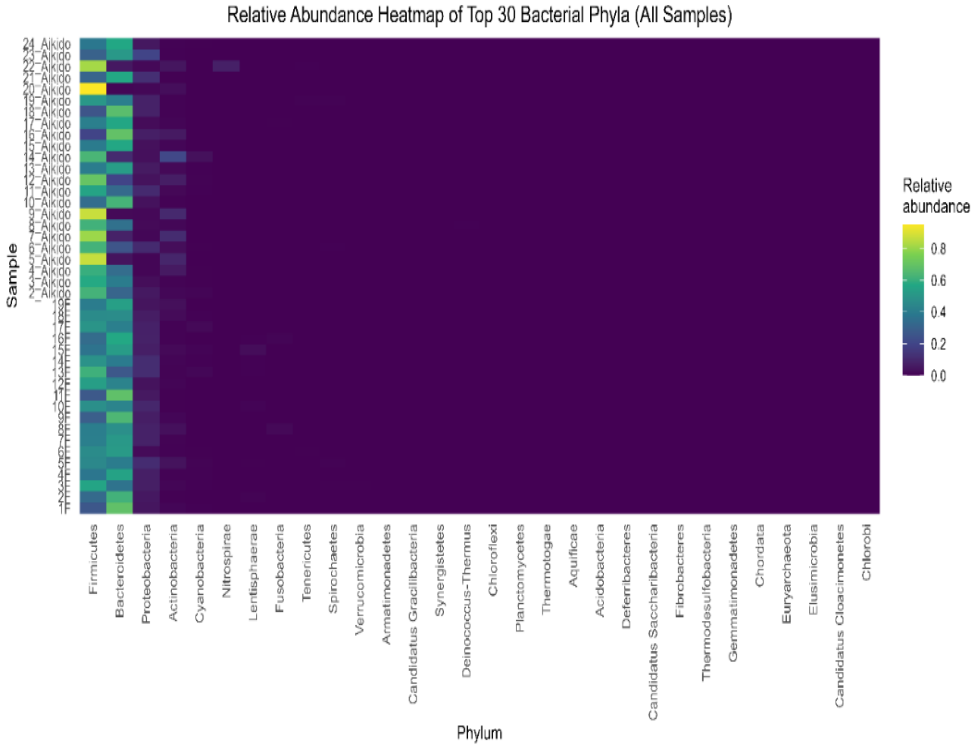


Figure 37. Phylum-level abundance heatmap and significant phyla. Heatmap of Z-score normalized phylum abundances, dominated by Firmicutes and Bacteroidetes (Ghaffar et al.,2026)

These results were concordant with the visual patterns observed in the genus- and phylum-level heatmaps, collectively indicating that Aikido and Tai Chi practitioners exhibit distinct gut microbial compositions across taxonomic resolutions. To further characterize the taxa potentially underlying the beta-diversity patterns, an additional heatmap was generated showing the 20 most prevalent genera across groups defined by the combined factors of

practice type and meditation level (Figure 38). Overall, compositional variation was apparent across both dimensions, suggesting that microbial profiles may differ according to both the movement practice and meditation depth. Across all groups, *Bacteroides* and *Faecalibacterium* were consistently among the most abundant genera, in line with their well-established prevalence in the human gut microbiota. Within Aikido practitioners, descriptive differences across meditation levels were observed for *Faecalibacterium***,** *Roseburia***,** *Ruminococcus***,** and *Butyricimonas*. Given prior literature linking *Faecalibacterium* and *Roseburia* to short-chain fatty acid production, these patterns may warrant further functional investigation; however, no functional profiling, metabolomics, or targeted assays were performed in the present study, and functional inference cannot be made. In the same Aikido strata, *Alistipes* and *Sutterella* appeared to show lower relative abundances at higher meditation levels.

Among Tai Chi practitioners, broadly similar descriptive patterns were noted, with higher meditation levels tending to show increased relative abundances of *Faecalibacterium* and *Roseburia***,** alongside practice-specific differences in other taxa relative to Aikido. For example, *Odoribacter* and *Parabacteroides* appeared more abundant in higher meditation-level Tai Chi groups than in the highest meditation-level Aikido group. Taken together, these observations suggest that both practice type and meditation level may be associated with distinct taxonomic profiles of the gut microbiota. These heatmap-based patterns were consistent with the beta-diversity structure observed in the PCoA ordinations and supported by ANOSIM results; nonetheless, they should be interpreted as descriptive and hypothesis-generating rather than as evidence of specific functional effects. Importantly, meditation-level strata within each practice group were markedly imbalanced, including single-participant cells, and therefore any interaction patterns should be regarded strictly as exploratory signals rather than confirmatory evidence of differential effects.

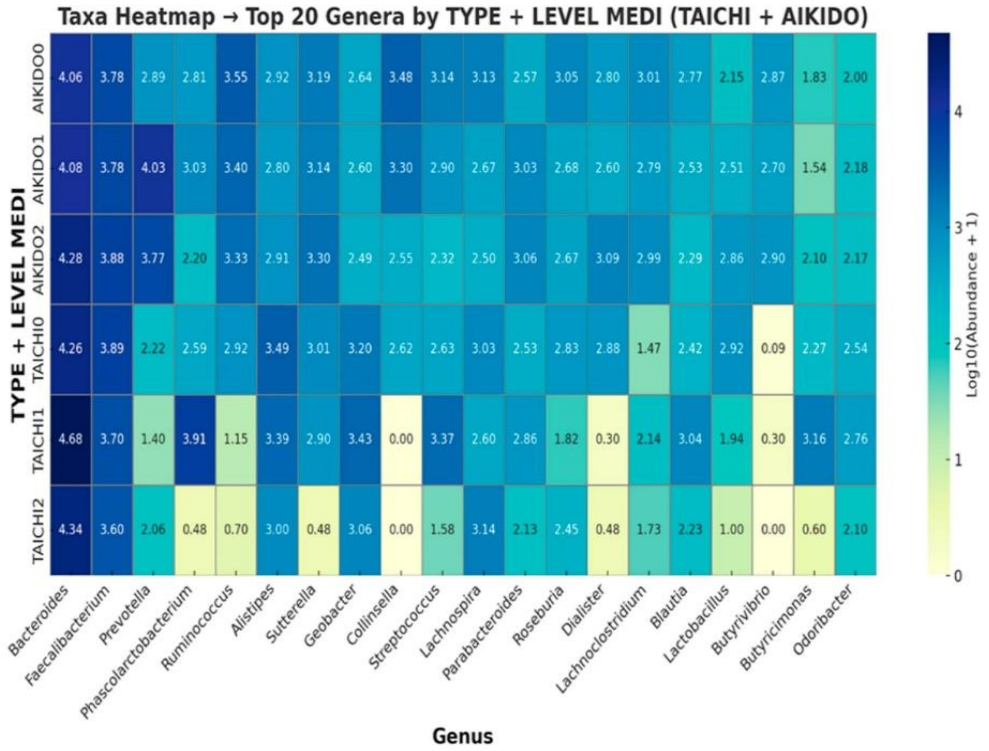


Figure 38. Heatmap of top 20 abundant genera by sport and meditation level. Visualization of community shifts with higher Faecalibacterium, Roseburia, and Butyricimonas in Aikido at higher meditation, and Odoribacter and Parabacteroides in Tai Chi at higher meditation (Ghaffar et al.,2026)

5.3.5 Phylogenetic Cladogram of Differentially Abundant Taxa Across Aikido and Tai Chi

Differential abundance analysis was performed to compare gut microbiota composition between Aikido and Tai Chi participants. The circular cladogram summarizes the phylogenetic relationships among the 50 most abundant genera, with node colours denoting group-associated enrichment: green indicates genera enriched in the Aikido group, whereas orange indicates genera enriched in the Tai Chi group (Figure 39). Genera enriched in Aikido were primarily affiliated with the Firmicutes and Bacteroidetes phyla and included *Faecalibacterium*, *Ruminococcus*, *Roseburia*, *Bacteroides*, and *Barnesiella*. Conversely, genera enriched in Tai Chi included *Prevotella*, *Collinsella*, *Lactobacillus*, *Dialister*, and *Phascolarctobacterium*.

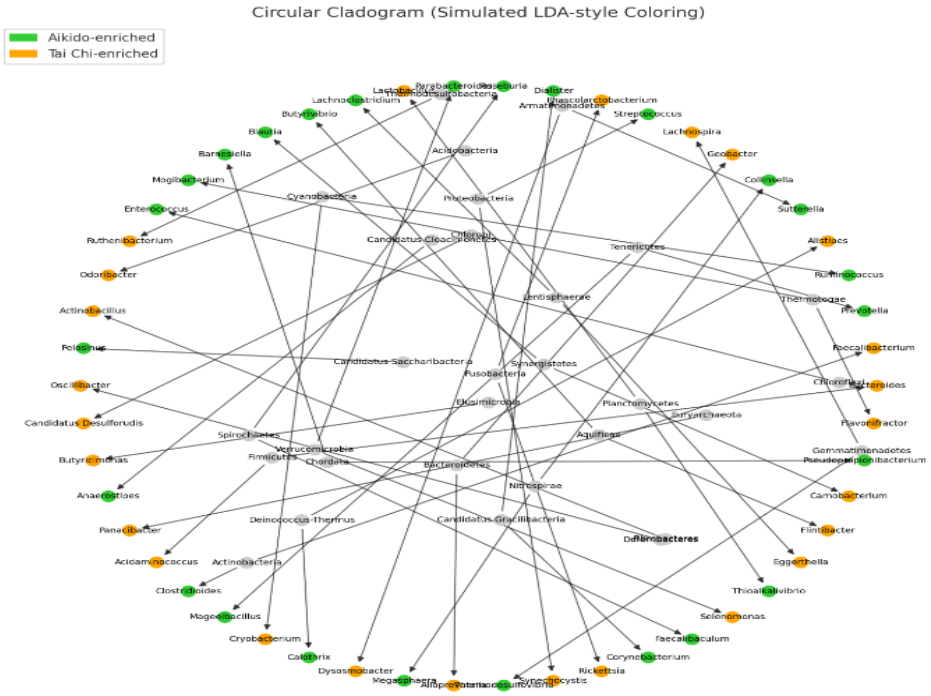


Figure 39. Phylogenetic cladogram of top 50 genera. Circular cladograms show phylogenetic relationships, with green nodes indicating genera enriched in Aikido (e.g., *Faecalibacterium, Roseburia*) and orange nodes indicating genera enriched in Tai Chi (e.g., *Prevotella, Collinsella)* (Ghaffar et al.,2026)

The bar plot summarizes the 20 genera exhibiting the largest log2 fold changes between Aikido and Tai Chi practitioners. *Faecalibacterium* and *Roseburia* were relatively more abundant in the Aikido group, whereas *Prevotella* and *Collinsella* were enriched in the Tai Chi group (Figure 40). Collectively, these results indicate that Aikido and Tai Chi practitioners display distinct gut microbiota profiles, driven by differential abundance of several key genera. Although the present study was not designed to identify causal determinants, these differences may plausibly reflect variation in lifestyle factors, dietary habits, and/or physiological adaptations associated with the two movement disciplines.

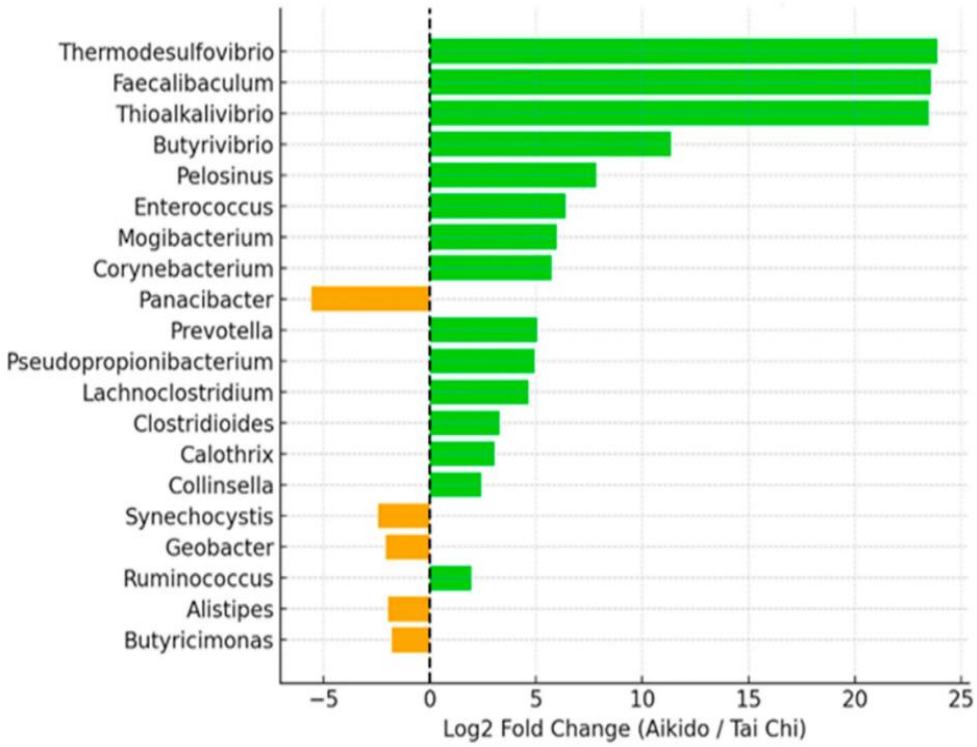


Figure 40. Differential abundance bar plot. Bar plot of the top 20 genera showing the largest log2 fold changes between groups, with Faecalibacterium and Roseburia enriched in Aikido and Prevotella and Collinsella enriched in Tai Chi (Ghaffar et al.,2026)

5.4 RESULTS OF CHARACTERIZATION OF ORAL MICROBIOTA AND SALIVARY BIOMARKERS IN TAI CHI PRACTITIONERS

5.4.1 Taxonomic Profiling of Oral Microbiota in Tai Chi Practitioners

To characterize the oral microbiota of Tai Chi practitioners, we performed taxonomic profiling of 16S rRNA gene amplicons from saliva samples. This profiling provided an in-depth view of the microbial community composition at various taxonomic levels, from phylum to genus, across different participants. We performed taxonomic profiling of the oral microbiota to assess the relative abundance of microbial phyla across the different samples. Our analysis revealed that *Firmicutes* and *Bacteroidetes* were the most dominant phyla in all samples, consistent with the typical composition of the human oral microbiome. *Proteobacteria* and *Actinobacteriota* were also present but at lower proportions, as shown in figure 41. Notably, there was variation in the relative abundance of these phyla between the samples, which suggests potential influences from factors such as diet, lifestyle, or exercise. Minor phyla like *Campylobacterota* and *Spirochaetota* were detected at lower levels, contributing to the overall diversity. The Unknown category indicates phyla that could not be confidently classified. These results highlight both the dominant phyla and the variability in microbial composition, suggesting that

exercise intensity and other factors may play a role in shaping the oral microbiota of Tai Chi practitioners.

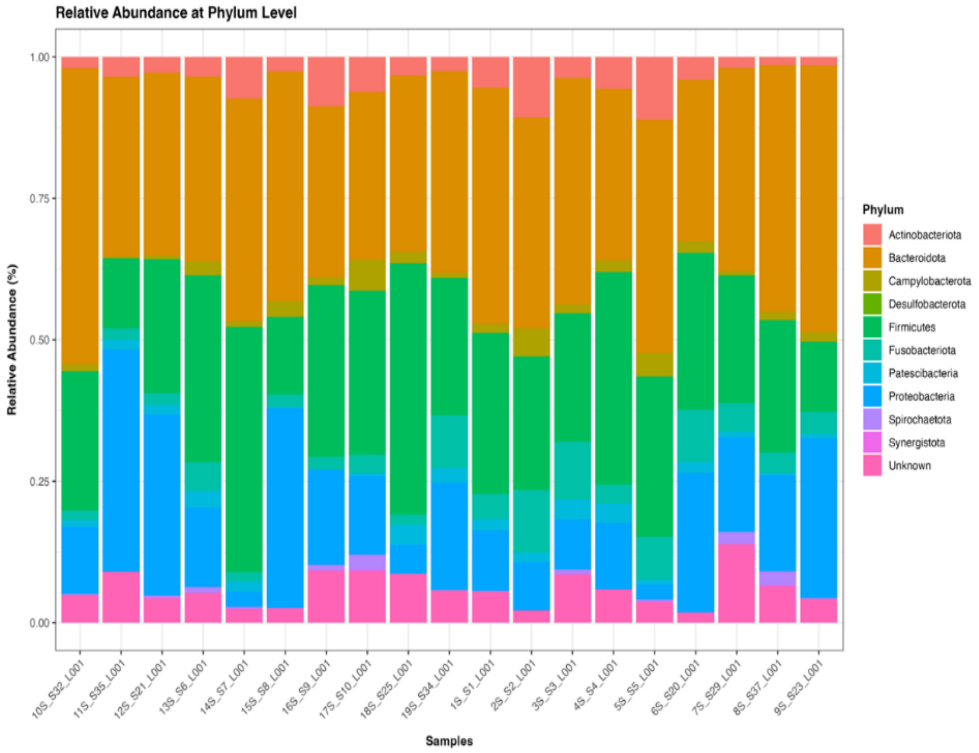


Figure 41. Relative abundance at phylum level

Further we performed taxonomic profiling of the oral microbiota at the family level to explore its composition across different samples (Figure 42). The analysis revealed that several families dominated the microbiome, with *Prevotellaceae*, *Neisseriaceae*, and *Streptococcaceae* showing the highest relative abundance across all samples. Notably, *Prevotellaceae* was consistently one of the most abundant families, highlighting its significant presence in the oral microbiota of Tai Chi practitioners. However, there was considerable variation in the relative abundance of other families, such as *Fusobacteriaceae* and *Bacteroidaceae*, suggesting differences in microbial composition based on factors like exercise intensity or individual lifestyle. These results indicate that while certain families remain prevalent across the cohort, other families exhibit dynamic abundance patterns, which could be influenced by physical activity or other environmental factors. The observed variations in family-level composition provide further insight into how exercise may shape the oral microbiome of Tai Chi practitioners.

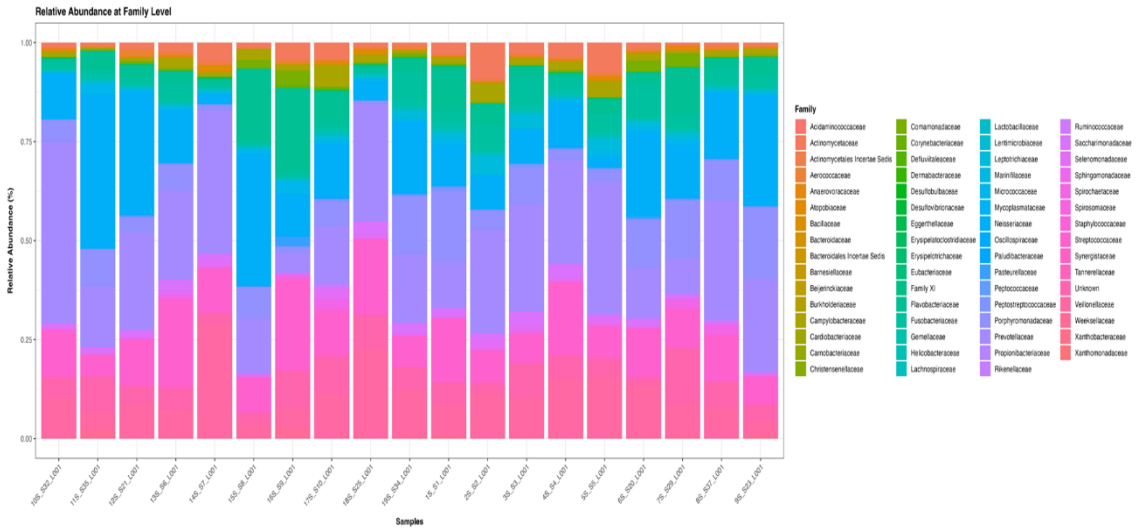


Figure 42. Relative abundance at Family level

Further we investigate the relative abundance of the top 10 microbial families, the data reveals the proportion of each family across different samples, with each colour representing a specific family. (figure 43) Among the top families, *Prevotellaceae*, *Neisseriaceae*, and *Streptococcaceae* consistently appear as the most abundant, accounting for most of the microbial community in most samples. These families, particularly *Prevotellaceae*, dominate the microbiome, suggesting their significant presence in the oral cavity.

Additionally, *Veillonellaceae, Porphyromonadaceae,* and *Flavobacteriaceae* are also present, but at lower relative abundances. The chart shows some variation in the composition of these families across different samples, indicating that while certain families are universally abundant, their relative proportions can fluctuate depending on factors such as exercise intensity, lifestyle, or diet. *Fusobacteriaceae, Actinomycetaceae,* and *Campylobacteraceae* also contribute to the microbial community but in smaller amounts. The presence of an Unknown category, shown in gray, suggests that some microbial families could not be confidently identified during the analysis.

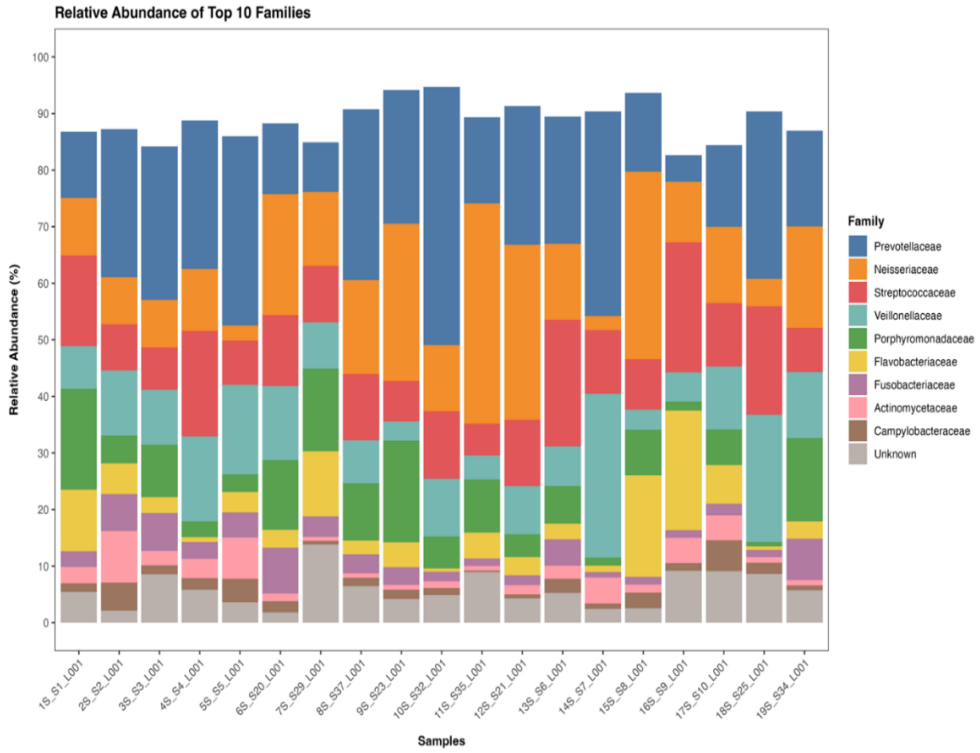


Figure 43. Relative abundance of top 10 Families

We performed taxonomic profiling at the genus level to further examine the oral microbiota composition across the samples. The most abundant genera include *Prevotella*, *Neisseria*, *Streptococcus*, and *Veillonella*, which are prevalent across all samples (figure 44). However, there are noticeable differences in the relative abundance of these genera between the samples, suggesting that the microbial community composition varies depending on factors such as exercise intensity and lifestyle. For instance, *Prevotella* and *Veillonella* were more abundant in certain groups, possibly reflecting differences in diet or physical activity. Meanwhile, genera such as *Streptococcus* and *Neisseria* were present at varying levels across the samples, contributing to the diversity of the microbiome. Minor genera, such as *Fusobacterium* and *Actinomyces*, also appeared but at much lower relative abundance.

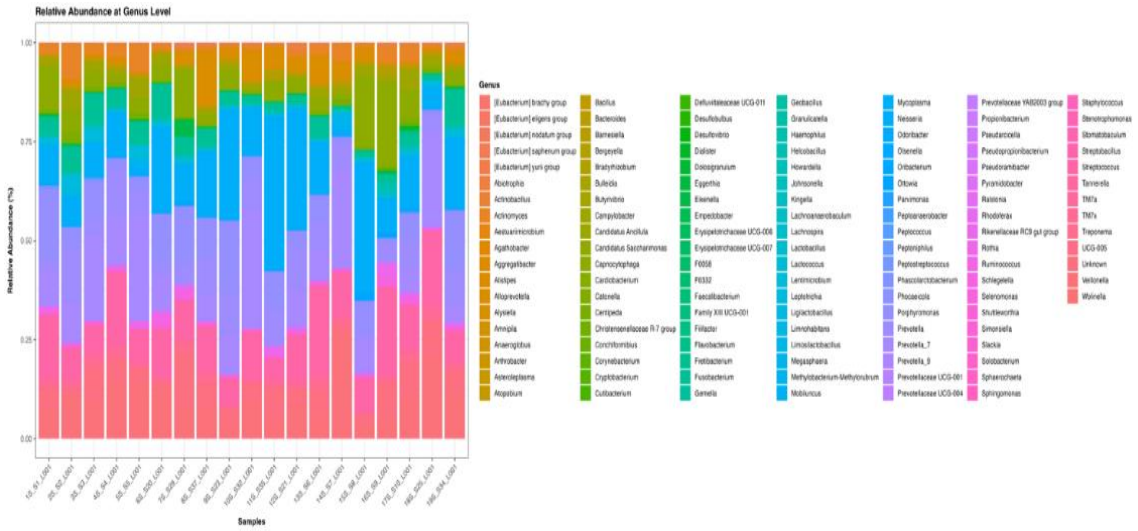


Figure 44. Relative abundance at Genus level

Further we investigate the top 10 genus, The dominant genera in this analysis include *Neisseria*, *Streptococcus*, *Prevotella_7*, and *Veillonella*, which make up a large proportion of the microbial community across all samples. *Neisseria* consistently appears as the most abundant genus, followed by *Streptococcus* and *Prevotella_7*, which are also present in significant amounts across all samples. (figure 45) *Porphyromonas*, *Prevotella*, and *Capnocytophaga* are also well-represented, but at lower relative abundances. Minor genera such as *Fusobacterium*, *Alloprevotella*, and *Unknown* are detected in smaller quantities, with *Unknown* accounting for a small portion of the total microbiome composition, possibly reflecting unidentified or poorly classified species.

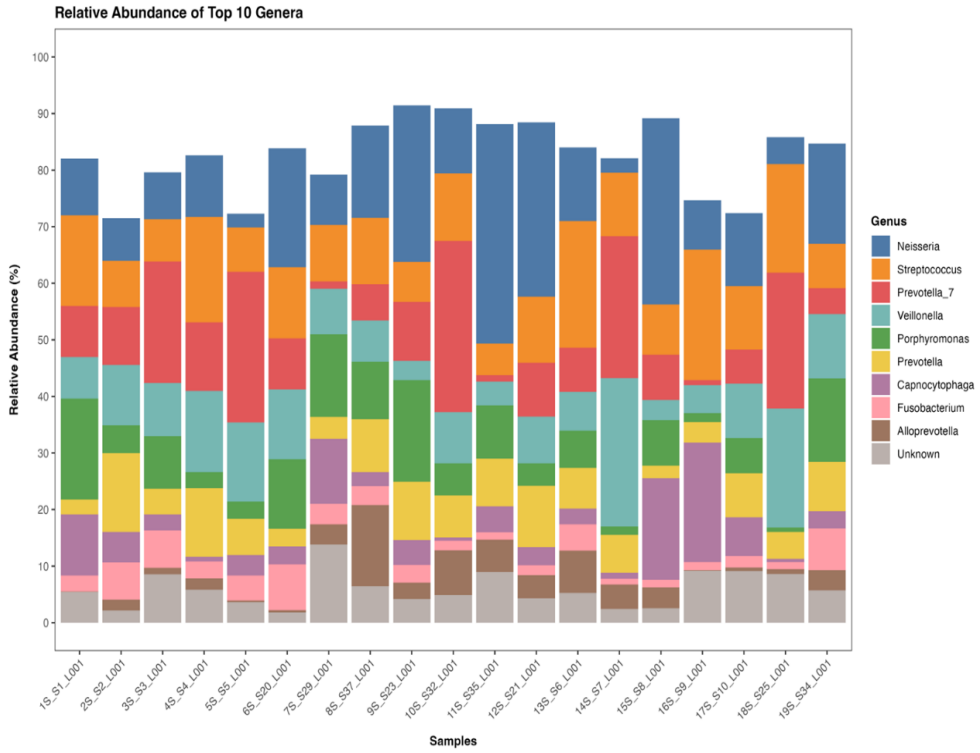


Figure 45 Relative abundance of top 10 Genera

To further understand the composition of the oral microbiota, we analysed the relative abundance of the top 20 genera, (figure 46) with a specific focus on how these genera vary across different Meditation Depth Index (MEDI) levels and Sport levels (Low, Medium, High). This genus-level analysis followed our previous examinations of the microbiome at the phylum and family levels, providing a more detailed view of microbial composition in Tai Chi practitioners. The bar chart displays the abundance of these genera across the different Meditation and Sport categories, where each bar represents an individual sample, and the color-coded segments correspond to individual genera. Our analysis reveals that dominant genera such as *Neisseria*, *Streptococcus*, and *Prevotella_7* remain prevalent across all groups, but there is notable variation in the relative abundance of other genera based on both Meditation and Sport levels. For instance, higher Sport levels and MEDI levels were associated with a more diverse microbial community, with an increase in the relative abundance of genera like *Leptotrichia*, *Rothia*, and *Gemella*. On the other hand, genera such as *Fusobacterium* and *Capnocytophaga* were more variable across the groups, suggesting that physical activity and meditation may play a role in shaping specific microbial populations. The presence of the *Unknown* category highlights genera that could not be confidently classified but constituted a minor proportion across all samples. These genus-level variations further emphasize how Meditation depth and Sport intensity influence the oral microbiome. The results suggest that both factors, along with other lifestyle elements, are associated with shifts in microbial

diversity, as revealed by our analysis at the genus level. This detailed view builds on our previous findings at the phylum and family levels, providing deeper insights into how lifestyle factors might directly impact the composition and health of the oral microbiota in Tai Chi practitioners.

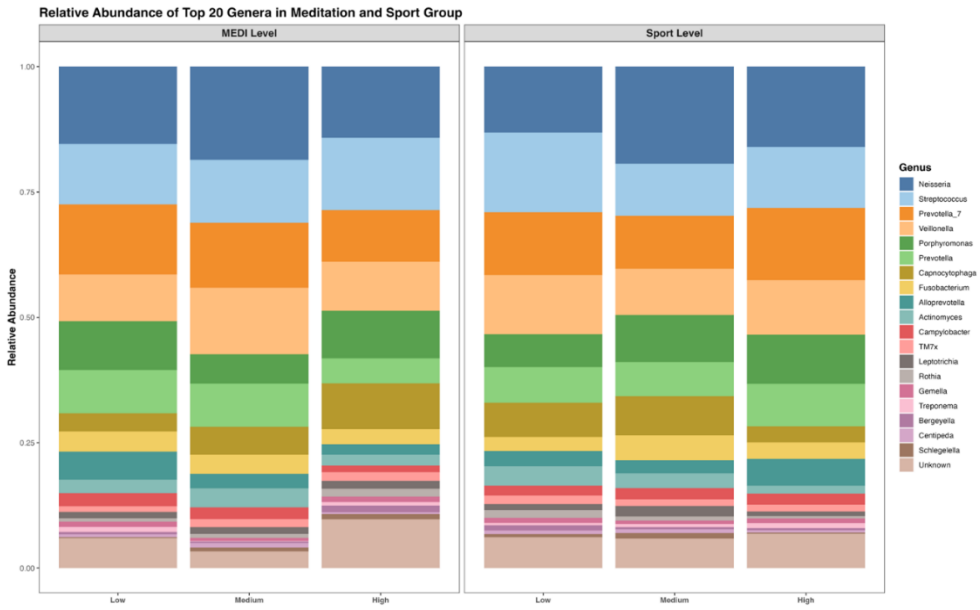


Figure 46. Genus-level relative abundance across Meditation Depth Index (MEDI) and Sport levels.

5.4.2 Alpha and Beta Diversity of Oral Microbiome

5.4.2.1 Alpha diversity indices

We performed alpha diversity analyses on the oral microbiome using Shannon and Simpson indices, examining the potential effects of different meditation levels (Low, Medium, High) and sports activity levels (Low, Medium, High). The Shannon index, which accounts for both the richness and evenness of the microbial communities, and the Simpson index, which emphasizes the dominance of particular species, were used to assess microbial diversity across the groups. The Shannon diversity index (Figure 47) was analysed to examine the variation in microbial diversity across the three Sport levels: Low, Medium, and High. As indicated by the Kruskal-Wallis test ($p = 0.995$), no significant differences in Shannon diversity were observed between the sport levels. While there was a slight visual trend suggesting higher diversity in the High sport group compared to the Low and Medium groups, the differences were not statistically significant. Similarly, the Simpson diversity index (Figure 47), which focuses on species dominance, revealed no significant differences across the sport levels (Kruskal-Wallis test, $p = 0.78$). The High sport level appeared to show a trend of slightly lower dominance, suggesting greater diversity, but again, this was not statistically significant. These results indicate that, at least within the scope of this study, sport activity levels did not have a clear

impact on the overall diversity or dominance of the oral microbiome.

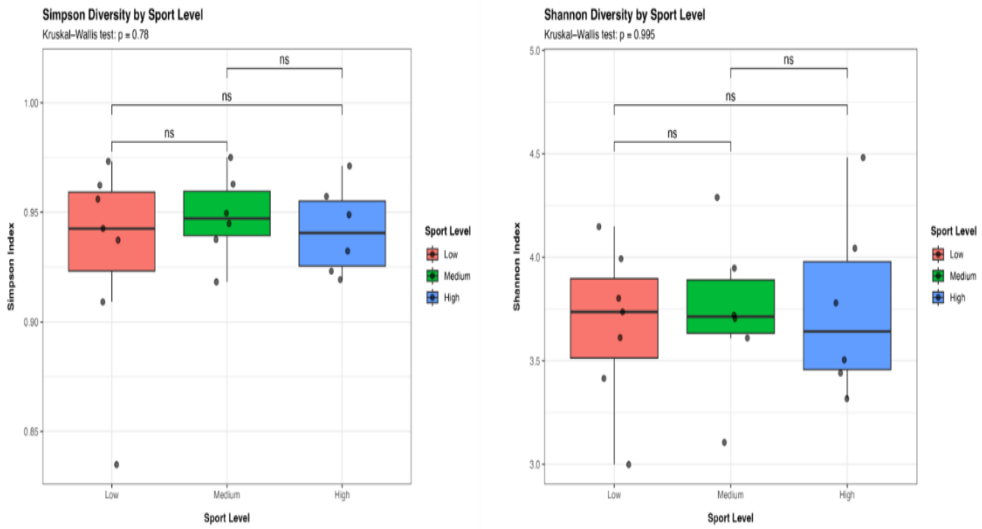


Figure 47. Shannon and Simpson diversity at Sports level.

For the Shannon diversity index (Figure 48), there was no significant difference in microbial diversity between the different meditation levels (Kruskal-Wallis test, p = 0.355). Although trends suggested a slightly higher diversity in the High MEDI group compared to the Low and Medium groups, these differences did not reach statistical significance, indicating that meditation depth did not appear to influence the overall richness or evenness of the oral microbiome in a meaningful way. Similarly, the Simpson diversity index (Figure 48) for the different meditation levels showed no significant differences (Kruskal-Wallis test, p = 0.702). The results indicated minor variations in the dominance of particular species, but again, these differences were not statistically significant across the three meditation groups. These findings suggest that while trends in microbial composition may exist, meditation depth did not show a significant effect on species dominance or diversity in the oral microbiome.

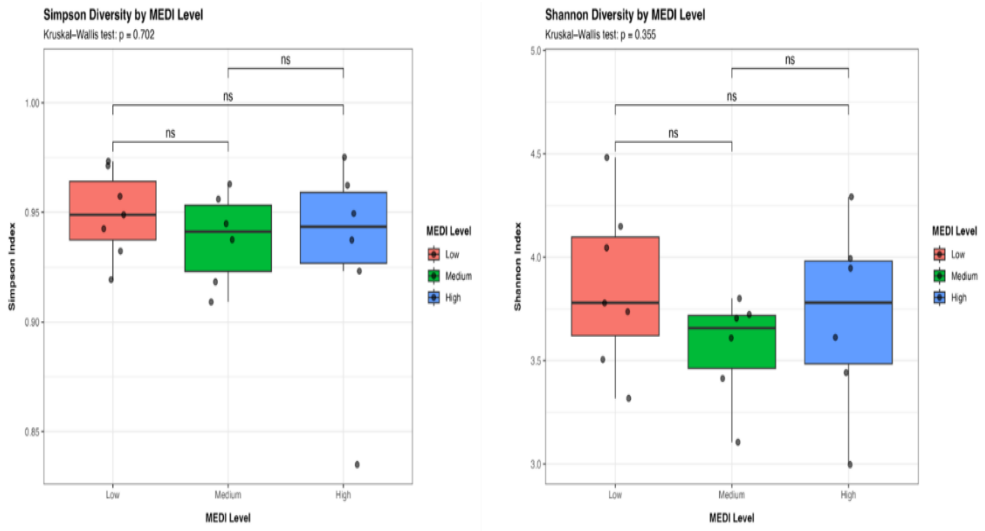


Figure 48. Shannon and Simpson diversity at MEDI level.

The analysis of Shannon diversity based on Gender and BMI revealed no significant differences between the groups.(Figure 49) For Gender, both male and female participants exhibited similar microbial diversity, with the Shannon index ranging between 3.5 and 4.0 (Kruskal-Wallis test, p = 0.14). Similarly, no significant differences were found across BMI categories (Normal weight, Overweight, and Underweight) with a similar Shannon index range (3.5 to 4.0) and a p-value of 0.28. These results suggest that neither gender nor BMI significantly affect the oral microbiome diversity in this cohort.

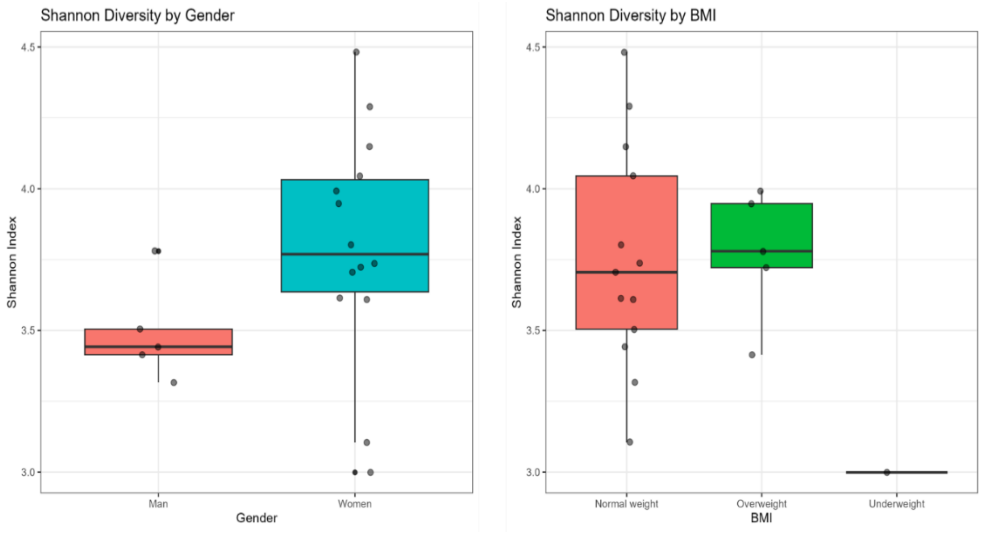


Figure 49. Shannon diversity by Gender and BMI.

5.4.2.2 Beta diversity indices

For beta diversity, we used Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity to examine the overall differences in microbial community composition between

different groups. The resulting plots for both meditation depth and sport levels reveal distinct clustering patterns. For Meditation Depth Levels (MEDI levels), the PCoA plot (Figure 50) illustrates the separation between the high, medium, and low MEDI level groups, with distinct clustering observed along the first principal coordinate (PCoA Axis 1, which explains 21.24% of the variance). While there is some overlap between the low and medium MEDI groups, the high MEDI group exhibits more separation, with a wider spread in PCoA Axis 2 (explaining 13.66% of the variance). These results suggest that individuals with higher meditation depth may have a distinct oral microbiota composition compared to those with lower meditation depth.

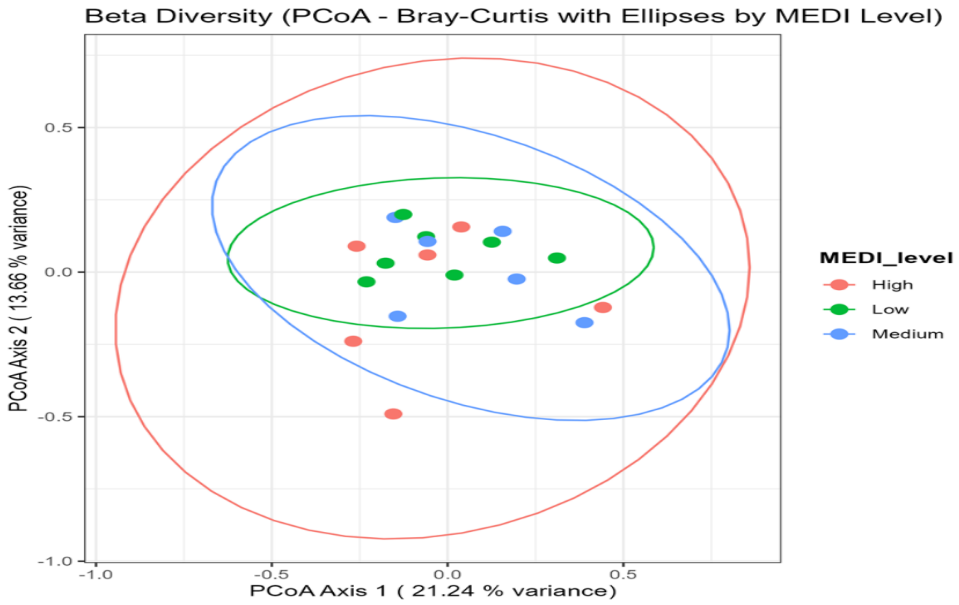


Figure 50. PCoA Bray Curtis with Ellipses by Meditation level

Similarly, for Sport Levels, the PCoA plot (Figure 51) also demonstrates distinct clustering across the high, medium, and low sport levels. Like the results from the MEDI groups, the high sport group shows a more spread-out distribution on both PCoA axes, while the medium and low sport level groups are more tightly clustered. This pattern further emphasizes that physical activity levels (e.g., low, medium, high) are associated with differences in oral microbiota composition. These results suggest that both meditation depth and sport level influence the beta diversity of the oral microbiota, leading to community compositions that are significantly distinct between the groups. The overall diversity of microbial communities, as reflected by the spread in the PCoA plots, may therefore be influenced by lifestyle factors such as physical activity and meditation practice.

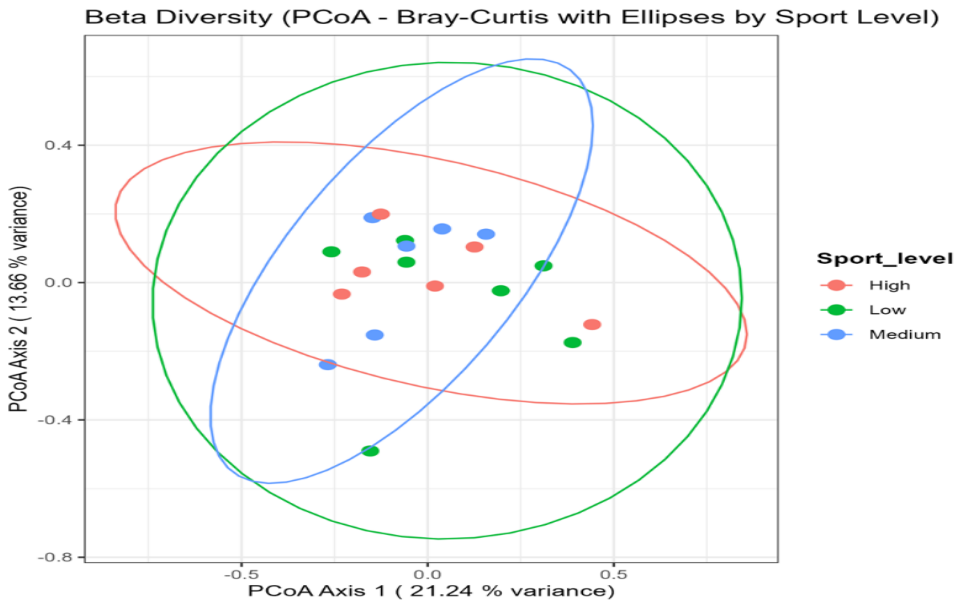


Figure 51. PCoA Bray Curtis with Ellipses by Sports level

5.4.3 Salivary Biomarkers

5.4.3.1 MMP9

Salivary MMP9 concentrations demonstrated significant differences across sport levels (Kruskal–Wallis p = 0.0187). Specifically, the high sport level group exhibited lower MMP9 concentrations compared to the medium and low sport level groups (Figure 52). Post-hoc analysis revealed a significant pairwise difference between the medium and high sport levels, indicating a potential inverse relationship between physical activity and inflammatory markers. However, no significant differences in MMP9 concentrations were observed across meditation levels (Kruskal–Wallis p = 0.127). Despite a visual trend indicating higher median MMP9 concentrations in participants with medium and high meditation levels, these findings did not reach statistical significance.

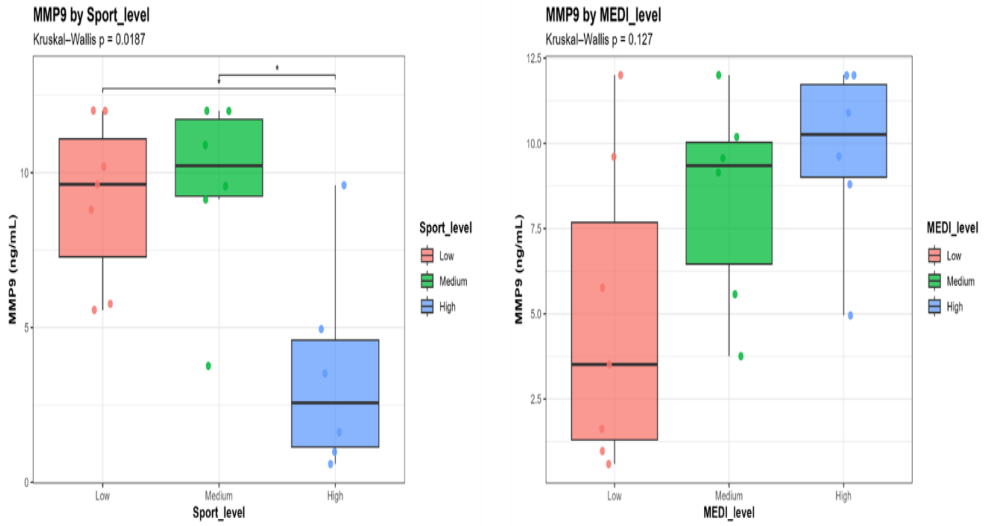


Figure 52. MMP9 Levels Across Different Meditation and Sport Levels

5.4.3.2 S100B

Salivary S100B concentrations did not exhibit significant differences across either meditation or sport levels (Figure 53). The Kruskal–Wallis test returned non-significant results for both meditation (p = 0.484) and sport levels (p = 0.933). There was considerable within-group variability, particularly in the low sport and low meditation groups, with no discernible monotonic trends across levels. This lack of significant difference may suggest that S100B is less sensitive to behavioral modulation in a generally healthy, physically active cohort.

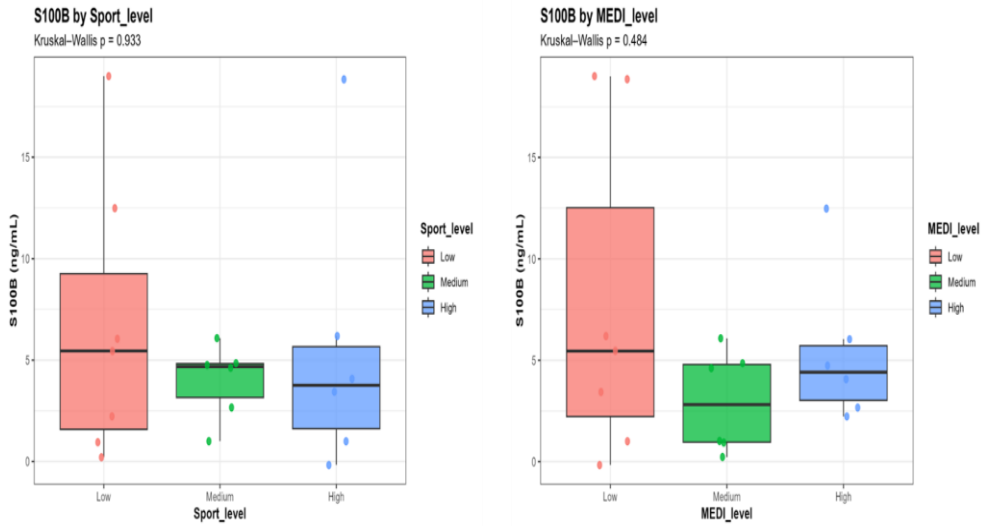


Figure 53. S100B Levels Across Different Meditation and Sport Levels

5.4.3.3 GFAP

Salivary GFAP concentrations were mostly below the lower limit of quantification (LLOQ = 0.625 ng/mL) in the majority of the samples (15/19). Consequently, GFAP was analyzed as a binary variable (detectable vs. below LLOQ). Despite a visual trend indicating higher detectability in participants with medium sport and meditation levels, statistical tests (Fisher's exact test) did not reveal significant associations between GFAP detectability and either sport level (p = 0.151) or meditation level (p = 0.805).(Figure 54 & 55)

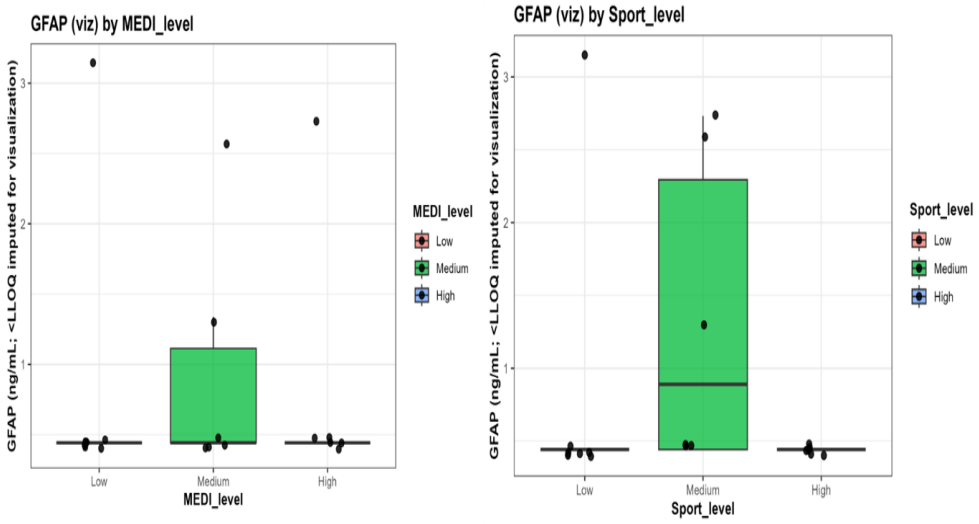


Figure 54. GFAP Levels Across Different Meditation and Sport Levels

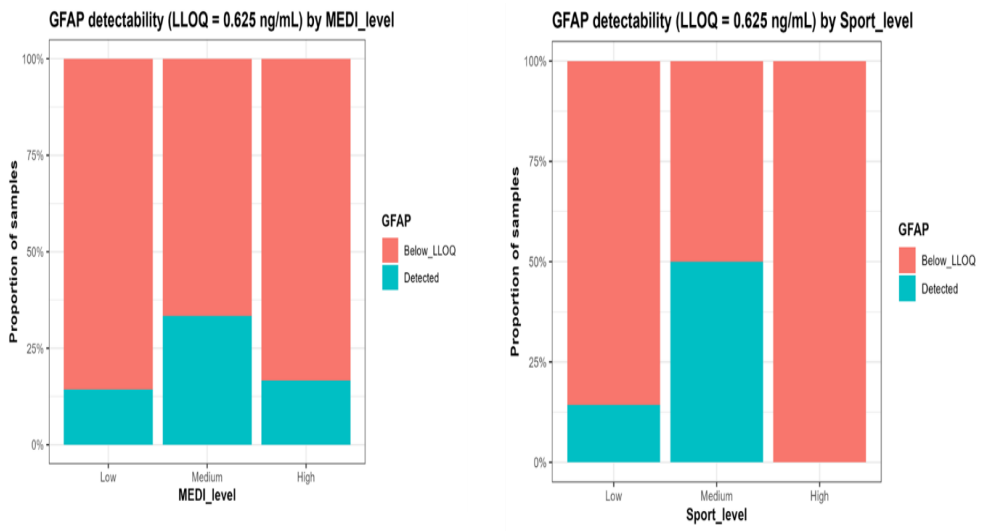


Figure 55. GFAP detectibility Across Different Meditation and Sport Levels

Overall Salivary MMP9 concentrations emerged as the most responsive biomarker to physical activity levels, suggesting that sustained high levels of physical activity may lead to the adaptive downregulation of inflammatory pathways. In contrast, meditation did not significantly influence MMP9 concentrations. S100B and GFAP showed limited sensitivity to either sport or meditation levels, with S100B exhibiting high variability and no clear pattern across the groups. Similarly, GFAP detectability was low across all groups, further suggesting that it may not be a reliable biomarker for inflammatory or neuro-glial activity in a healthy cohort, particularly when measured in saliva.

5.5 RESULTS OF MAGS ANALYSIS

5.5.1 Overview of cohorts and exercise-intensity stratification

Six publicly available shotgun metagenomic cohorts, each representing distinct sports modalities, were integrated in this study, including runners, rugby players, cricketers, cyclists, rowers, and sedentary controls. To facilitate cross-cohort comparisons of microbiome composition and genome-resolved features across different exercise intensity categories, the samples were grouped according to an ACSM-based classification scheme. Based on this classification, the data were further divided into five groups: A1, B2, C1, C3, and S, with group S representing the sedentary controls. The group A1 contains 24 samples, group B2 contains 40 samples, group C1 contains 294 samples, group C3 contains 130 samples and group S sedentary contain 165 samples as shown in Table 12.

Table 12. Characteristics of the athlete metagenomic cohort.

Study accession	Participant Type	Sport	Sample	Data Source	Sequencing Platform
PRJEB46398	Athlete	Runners	240	NCBI	Shotgun
PRJEB15388	Athlete + Controls	Rugby + sedentary	86	NCBI	Shotgun
PRJEB20054	Controls	Sedentary	116	NCBI	Shotgun
PRJEB28338	Athlete	Cricket	24	NCBI	Shotgun
PRJNA305507	Athlete	Cyclist	88	NCBI	Shotgun
PRJNA472785	Athlete	Rower + Runners	87	NCBI	Shotgun

5.5.2 Read-based taxonomic profiling across exercise groups

Read-based taxonomic profiling (MetaPhlAn/Kraken-based summaries) indicated that dominant gut microbial taxa differed in relative abundance across exercise-intensity groups. Across cohorts, distinct abundance patterns were observed for key gut species, supporting the presence of exercise-associated compositional signatures and motivating subsequent genome-resolved reconstruction to refine strain- and species-level interpretation.

5.5.2.1 Alpha Diversity Across Exercise-Intensity Groups

Alpha diversity differed across ACSM-defined categories, with the highest diversity observed

in high-intensity dynamic athlete groups (C1/C3), while lower-intensity athlete groups and sedentary controls showed reduced diversity. Alpha diversity analyses revealed clear differences in within-sample microbial diversity across exercise-intensity groups. When assessed using Shannon and Simpson indices, high-intensity dynamic athlete groups (C1 and C3) consistently exhibited the highest alpha diversity**,** whereas sedentary controls (S) showed the lowest diversity values (Figure 56).

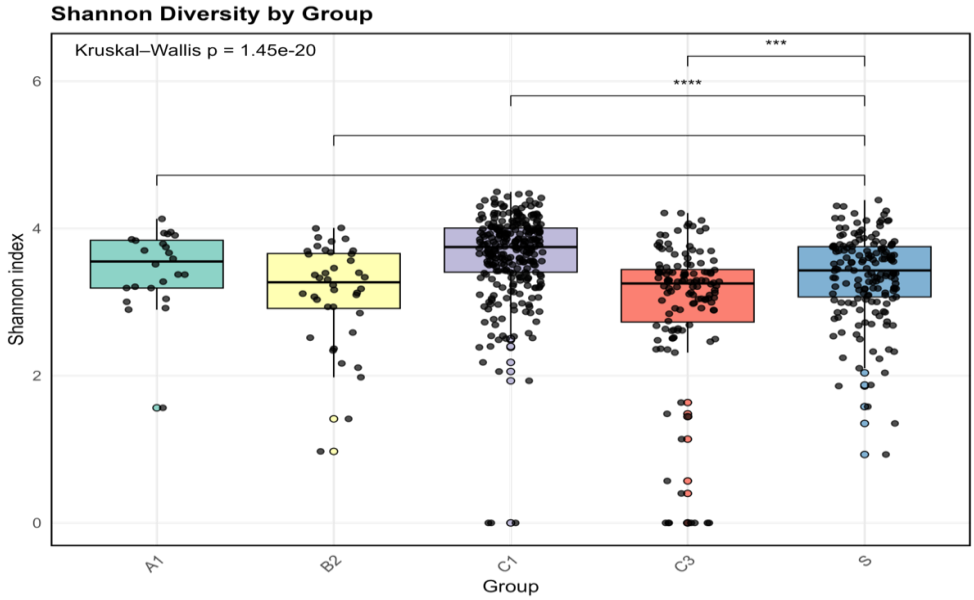


Figure 56. Shannon diversity across sports classification groups

Intermediate diversity levels were observed in low- and moderate-intensity athlete groups (A1 and B2), indicating a graded pattern across the exercise-intensity spectrum. Boxplot visualizations (Figure 57) demonstrated a progressive increase in diversity from sedentary individuals to high-intensity athletes, with reduced inter-individual variability within the C1 and C3 groups compared with sedentary controls. Statistical testing using the Kruskal–Wallis test confirmed significant differences in alpha diversity across ACSM-defined categories ($p < 0.05$). Significant differences between groups are indicated by the asterisks above the plots. The results demonstrate that group S (sedentary controls) and group C1 show the most prominent differences in diversity, with C1 exhibiting significantly higher diversity compared to S. This suggests that exercise intensity has a notable effect on microbial diversity, with higher-intensity groups (such as C1) showing increased diversity relative to sedentary individuals (S) and lower intensity groups (C3).

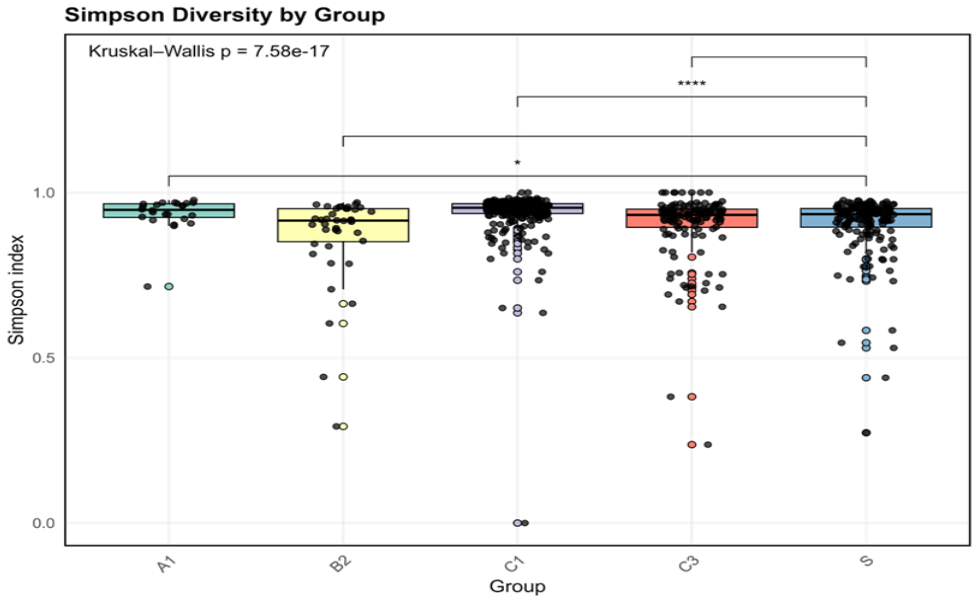


Figure 57. Simpson diversity across sports classification groups

The overall alpha diversity differs across groups, and the clearest pairwise separations are driven by the sedentary controls (S) and the C1 runners, which show the most statistically significant differences relative to other groups. In other words, while several groups overlap, S and C1 are the ones that most consistently 'pull apart' from the rest in these diversity metrics

5.5.2.2 Beta Diversity Across Exercise-Intensity Groups

The analysis of beta diversity indices across different exercise-intensity groups reveals substantial insights into the microbial community structure as shown in Figure 58. Beta diversity measures the dissimilarity between microbial communities across groups, offering a comprehensive view of the variability in microbiome composition influenced by exercise. The Principal Coordinates Analysis (PCoA) of beta diversity shows a clear distinction in the microbial communities of athletes and sedentary individuals.

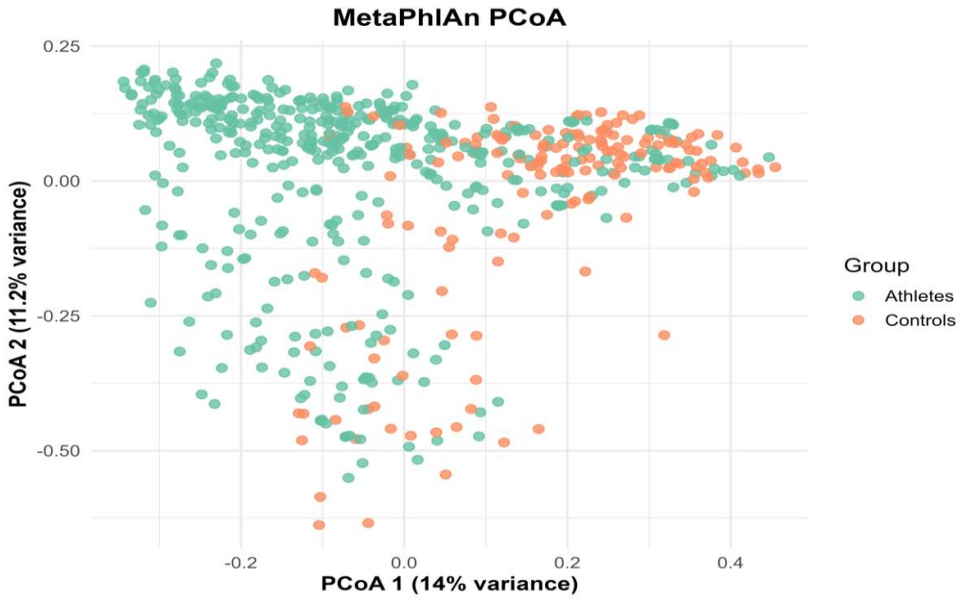


Figure 58. Community level microbiome differences across Athletes and sedentary controls

Moreover, the athletes regardless of their specific sport or exercise intensity, tend to exhibit distinct microbial profiles compared to sedentary controls. This separation along the primary axes of variation (PCoA1 and PCoA2) emphasizes the significant impact that exercise intensity has on the microbial community structure.

This PCoA plot shows the microbial community structure across different exercise intensity groups, represented by exercise categories A1, B2, C1, C3, and sedentary controls (S). The axes of the plot represent the two principal coordinates, PCoA1 (14% variance) and PCoA2 (11.2% variance), which capture the largest sources of variability in the microbial community composition. The plot demonstrates clear differentiation between groups, with each group clustering distinctly based on their exercise intensity. The higher-intensity groups (A1, B2, C1) show noticeable separation, with a wider spread across the PCoA1 axis. On the other hand, the sedentary group (S) and C3 group (low-intensity) are more tightly clustered, indicating less variability in their microbial communities compared to the higher-intensity groups.as shown in Figure 59 This suggests that exercise intensity influences the structure of the microbiome, with higher-intensity exercise leading to more pronounced differences in microbial composition.

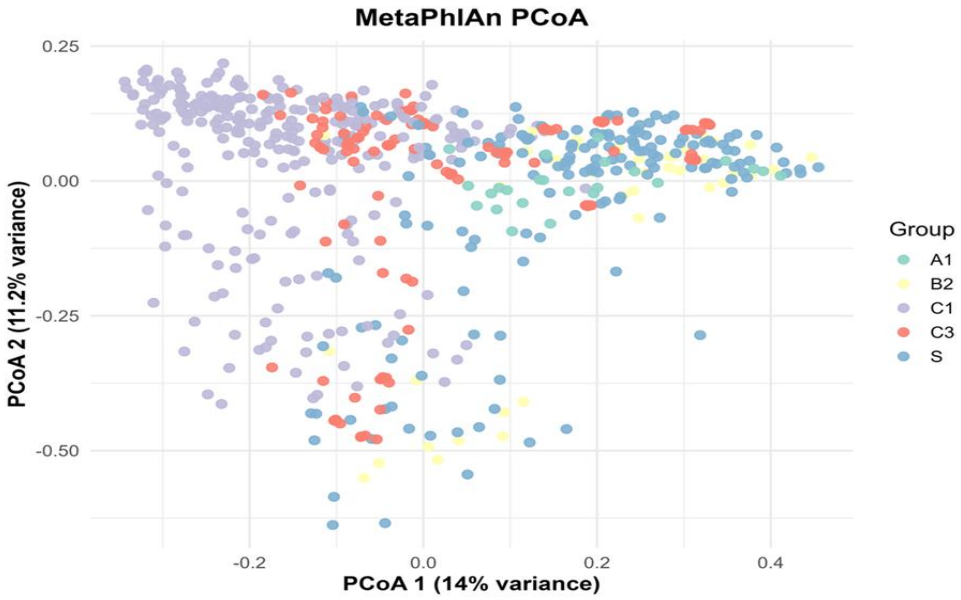


Figure 59. Community level microbiome differences across sports classification groups

The microbial community clustering across different exercise-intensity groups using Principal Coordinates Analysis (PCoA) and includes two separate representations: group ellipses and group vectors. The clustering of the exercise-intensity groups (A1, B2, C1, C3, and S) on the PCoA plot, (Figure 60) with each group represented by different colors. The ellipses surrounding each group indicate the distribution and spread of the microbial communities within that group. Notably, the higher-intensity groups (A1 and B2) display more distinct and separated ellipses compared to the lower-intensity (C3) and sedentary (S) groups, which suggests more variation within the lower-intensity groups. The clear separation between groups reflects how exercise intensity influences microbial composition, with higher-intensity groups tending to exhibit greater differentiation in community structure.

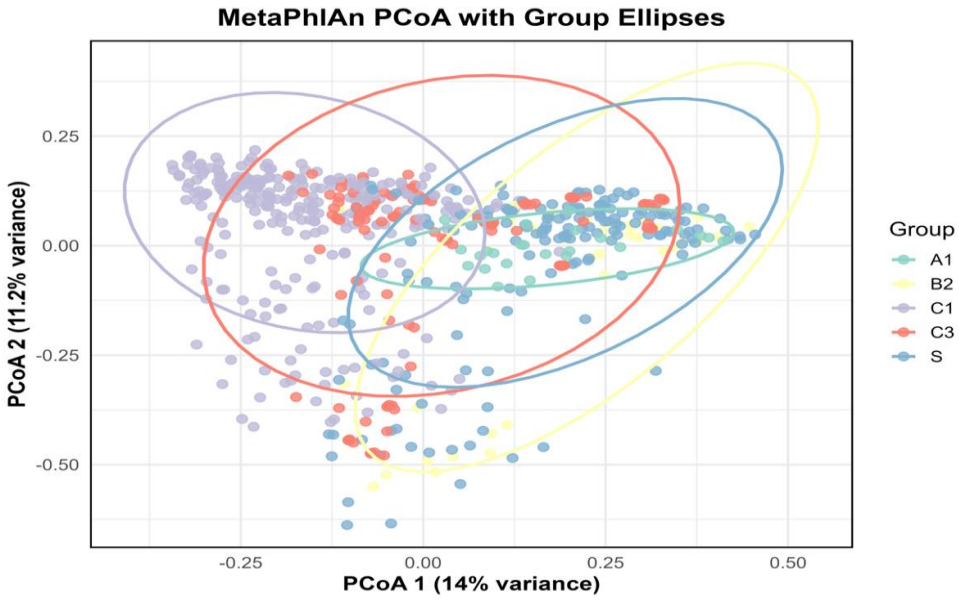


Figure 60. MetaPhlAn PCoA with Group Ellipses :Microbial Community Clustering Across Exercise-Intensity Groups

The group vectors to illustrate the directionality and relationships between the microbial communities of different exercise-intensity groups (Figure 61). The vectors show that group C1 (the highest intensity) is positioned distinctly along the negative PCoA1 axis, while groups A1 and B2 show a gradient towards the positive side of PCoA1. This suggests a directional trend in microbial community structure with respect to exercise intensity, where increasing exercise intensity (C1 → B2 → A1) correlates with a shift in microbial composition, as shown by the orientation of the vectors. High-intensity athlete groups (C1 and C3) formed more compact and clearly separated clusters**,** whereas sedentary controls exhibited broader dispersion**,** reflecting greater inter-individual variability. Low- and moderate-intensity athlete groups occupied intermediate positions within the ordination space, partially overlapping with both high-intensity athletes and sedentary controls. This spatial arrangement was consistent across ordination methods and reflected a continuum rather than discrete separation between exercise categories.

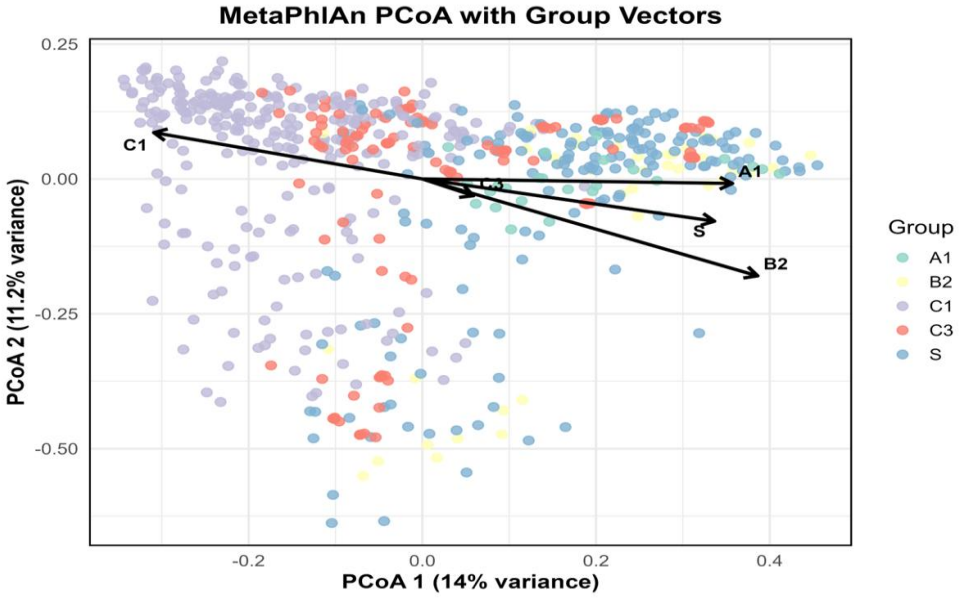


Figure 61. MetaPhlAn PCoA with Group Vectors : Microbial Community Clustering Across Exercise-Intensity Groups

These plots emphasize that higher-intensity exercise is associated with more distinct and differentiated microbial communities compared to sedentary or lower-intensity exercise groups. The clustering and vector orientations reveal how exercise intensity shapes microbial diversity, with significant changes in community structure as exercise intensity increases.

To assess the impact of exercise intensity on microbial community structure, statistical evaluations were performed to examine both the differences between groups and the variability within groups. The following figures present the results of these analyses, focusing on the differentiation in microbial composition and the dispersion of microbial communities across exercise-intensity groups. Statistical evaluation using PERMANOVA (Adonis) confirmed that exercise-intensity classification explained a significant proportion of variance in microbial community composition ($p < 0.05$) (Figure 62). PairwiseAdonis analyses further identified significant differences between high-intensity athlete groups and sedentary controls, while comparisons among athlete subgroups showed more modest effect sizes. This Pairwise PERMANOVA effect sizes (R^2), which assess the statistical differences in microbial community composition between exercise-intensity groups. Higher R^2 values indicate greater differentiation in microbial communities between the groups. Significant differences (denoted by asterisks) are observed between the high-intensity groups (A1, B2, C1) and sedentary (S) or lower-intensity groups (C3), with A1 showing the highest differentiation from other groups. These results suggest that higher-intensity exercise leads to more distinct microbial compositions compared to lower-intensity exercise and sedentary controls.

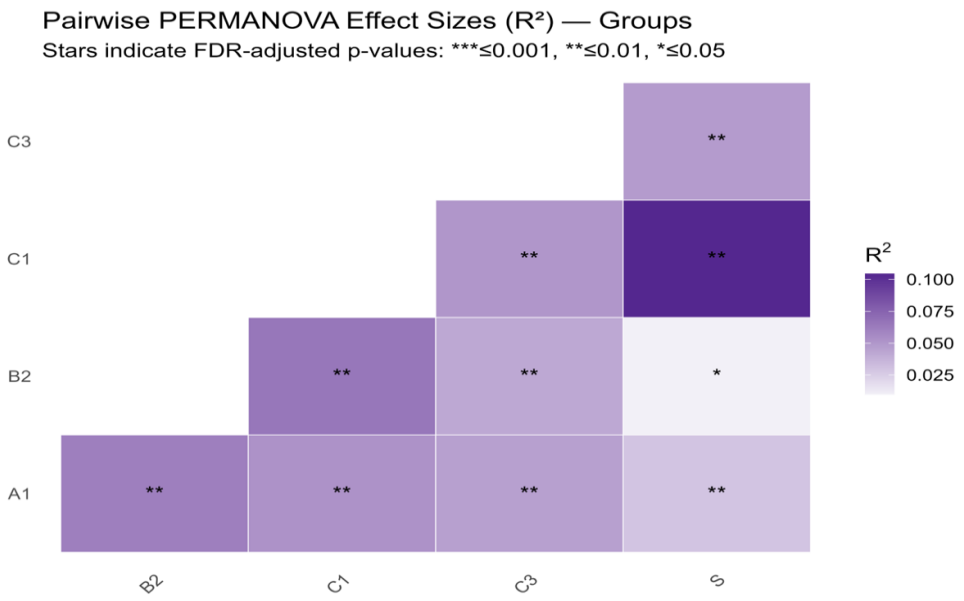


Figure 62. Pairwise **PERMANOVA** effect sizes (R²) for comparisons between exercise-intensity groups

This Beta-dispersion (Bray-Curtis) plot, which evaluates the variability of microbial communities within each exercise-intensity group. The distance to centroid indicates the degree of variability, with higher values representing greater dispersion. Higher-intensity exercise groups such as A1 and B2 show lower dispersion, indicating more homogeneous microbial communities (Figure 63). In contrast, S (sedentary) and C3 (low-intensity) groups exhibit higher dispersion, suggesting greater variability within these groups. This highlights the influence of exercise intensity on the consistency of microbial community composition.

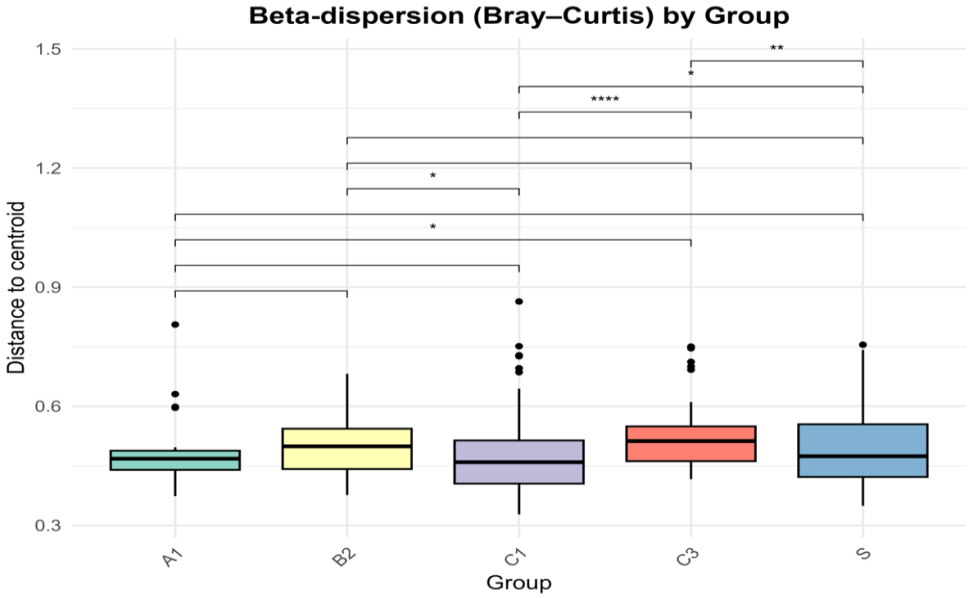


Figure 63. Beta-dispersion (Bray-Curtis) by Group

Importantly, beta-dispersion patterns indicated lower dispersion within high-intensity groups**,** suggesting more homogeneous microbial community structures among these athletes. In

contrast, sedentary controls displayed the highest dispersion, indicating greater variability in microbiome composition.

Taken together, alpha- and beta-diversity analyses demonstrate that gut microbiome diversity and structure differ across exercise-intensity groups. High-intensity dynamic athletes are characterized by higher within-sample diversity and more homogeneous between-sample community structure**,** whereas sedentary individuals show reduced diversity and increased inter-individual variability. These diversity patterns are consistent across multiple metrics and are statistically supported by both univariate and multivariate analyses.

5.5.2.3 Microbial Composition and Abundance Patterns Across Exercise-Intensity Groups

To further investigate the microbial composition and its variations across the groups, we performed a heatmap analysis, which allowed us to identify species abundance patterns and compare the gut microbiota of group C1, S, and the remaining groups (Figure 64). The heatmap analysis reveals significant differences in the gut microbiota composition between group C1 and group S, with distinct species abundances observed in each group. In group C1, the microbiome is dominated by species such as *Faecalibacterium prausnitzii*, *Roseburia intestinalis*, and *Bacteroides uniformis*, which are generally associated with beneficial gut health due to their roles in fermenting fibre and producing short-chain fatty acids. These species appear with higher relative abundance, suggesting a favourable and health-promoting microbial profile in this group. In contrast, group S exhibits a more diverse microbial composition, with an increased abundance of species like *Alistipes putredinis*, *Bacteroides thetaiotaomicron*, and *Prevotella copri*. These species may be linked to a more varied metabolic environment, possibly indicating a less stable gut microbiome. The observed differences between groups C1 and S suggest that group C1 may benefit from a more balanced microbiota, potentially influencing better health outcomes.

When comparing these groups to the remaining groups, such as A1, B2, and C3, notable variations in species abundance are also present. For instance, group A1 shows high abundance of *Segatella copri* and *Faecalibacterium prausnitzii*, which overlap with group C1, suggesting a similarly beneficial microbiome composition. Group B2, on the other hand, harbours species like *Bacteroides plebeius* and *Prevotella copri*, and group C3 is characterized by a diverse set of species, reflecting distinct dietary or environmental influences. Overall, while groups C1 and A1 appear to share more beneficial microbial species, groups S, B2, and C3 exhibit greater microbial diversity, which may be associated with different metabolic pathways or health implications. These results underscore the complexity of the human gut microbiome and its

potential role in shaping health outcomes across different populations.

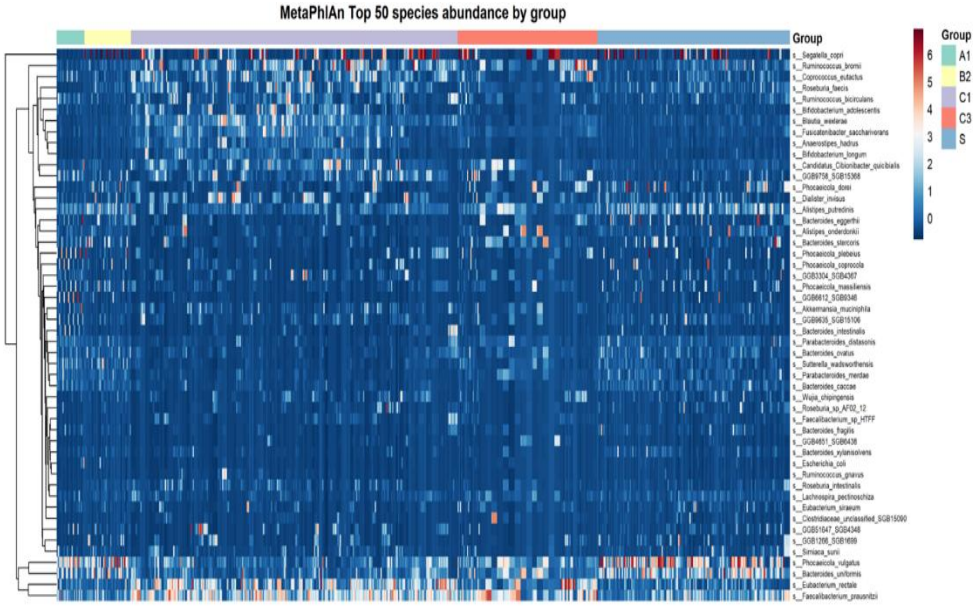


Figure 64. Heatmap of the relative abundance of the top 50 dominant gut microbial species across different groups (C1, S, A1, B2, C3). Color intensity represents the abundance levels, with warmer hues indicating higher abundance and cooler hues indicating lower abundance. The hierarchical clustering of species and samples is based on similarities in microbial composition, allowing for visual comparison of gut microbiota profiles across the groups

5.5.3 MAG recovery and quality metrics

Genome-resolved reconstruction across all cohorts yielded more than 11,500 MAGs**,** including approximately 4,887 high-quality (HQ) MAGs, providing a substantial genome catalogue for comparative analyses. MAG recovery differed markedly by binning strategy: MetaWRAP produced substantially more HQ MAGs **(**4,887 HQ MAGs**)** than MetaBAT **(**2,004 HQ MAGs**),** indicating higher reconstruction yield when refinement and integration steps were applied. The distribution of HQ and MQ MAGs varied across cohorts, reflecting differences in cohort design and sequencing characteristics. Only HQ MAGs were retained for downstream taxonomic and comparative analyses (Figure 65).

Accession No	Sport	Total MAGS	HQ MAGS	MQ MAGS
PRJEB46398	Running	4251	1621	2393
PRJEB15388	Rugby + sedentary	1679	731	803
PRJEB20054	Sedentary	3608	1741	1743
PRJEB28338	Cricket	193	47	107
PRJNA305507	Cycling	1341	575	648
PRJNA472785	Rowing+ Running	519	172	270
		11591	4887	5964

Figure 65. Distribution of MAGS across cohorts

The distribution of high-quality (HQ) and medium-quality (MQ) metagenome-assembled genomes (MAGs) across various datasets shown in figure 66, represented by their respective accession numbers. The red bars correspond to HQ MAGs, while the teal bars represent MQ MAGs. As shown, the dataset with accession number *PRJEB46398* contains the highest number of both HQ and MQ MAGs, while *PRJEB28338* contains relatively fewer MAGs overall. This comparison highlights the variability in MAG quality and quantity across different datasets.

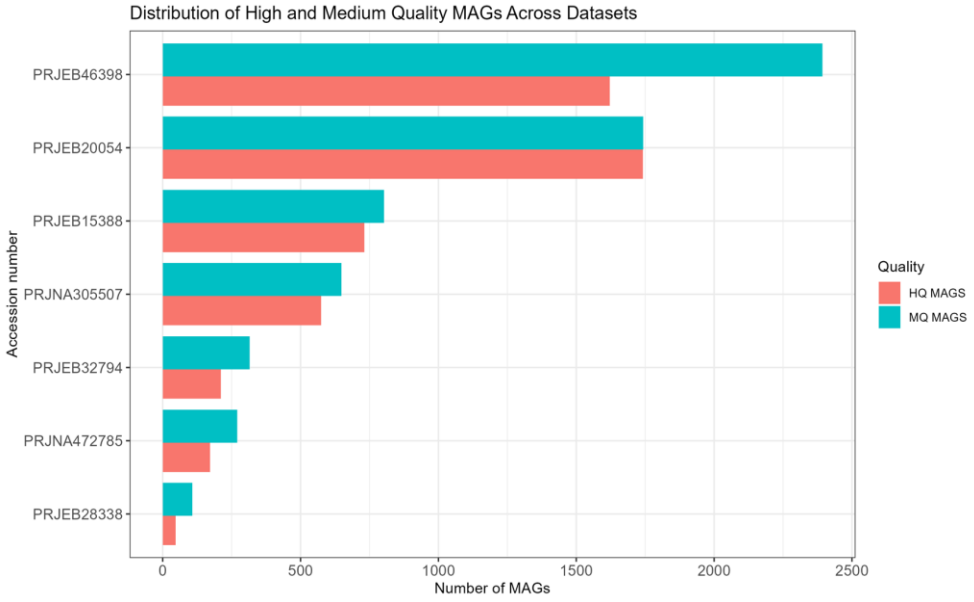


Figure 66. Distribution of high and medium quality MAGS across datasets

5.5.3.1 MetaWRAP and MetaBAT Results: Comparison of High-Quality Cohort Groups

To assess the microbial composition and distribution across different cohort groups, we

compared two different binning techniques MetaWRAP and MetaBAT. Our primary focus was on MetaWRAP, as it provides a more comprehensive and accurate assessment of high-quality metagenome-assembled genomes (HQ MAGs).

We examined the top 20 high-quality MAGs identified (Figure 67) by MetaWRAP across the different cohort groups (A1, B2, C1, C3, and S). The cohort groups were categorized by sports activity (cricket, cycling, rugby, running, and sedentary). This figure presents the distribution of HQ MAGs for each species within these cohorts, offering insight into how species vary across the different activity levels. It shows the distribution of the top 20 high-quality metagenome-assembled genomes (HQ MAGs) per species across the different cohort groups (A1, B2, C1, C3, and S). The number of MAGs for each species is represented on the x-axis, while the y-axis lists the species identified across the cohorts. The cohorts are color-coded according to the type of sport: cricket (teal), cycling (light yellow), rowing (purple), rugby (red), running (blue), and sedentary (orange). From this visualization, we can see that species like *Alistipes putredinis* and *Agathobacter rectalis* are more abundant in specific cohorts (e.g., *Alistipes putredinis* is prevalent in group A1, marked by cricket players). Additionally, certain species show high numbers of MAGs across multiple cohorts, such as *Ruminococcaceae intestinalis*, which is common across the cycling (B2) and sedentary (S) groups. This figure highlights the differences in microbial diversity and abundance between the various activity levels, providing insight into how different sports or sedentary behavior are associated with specific gut microbiota compositions.

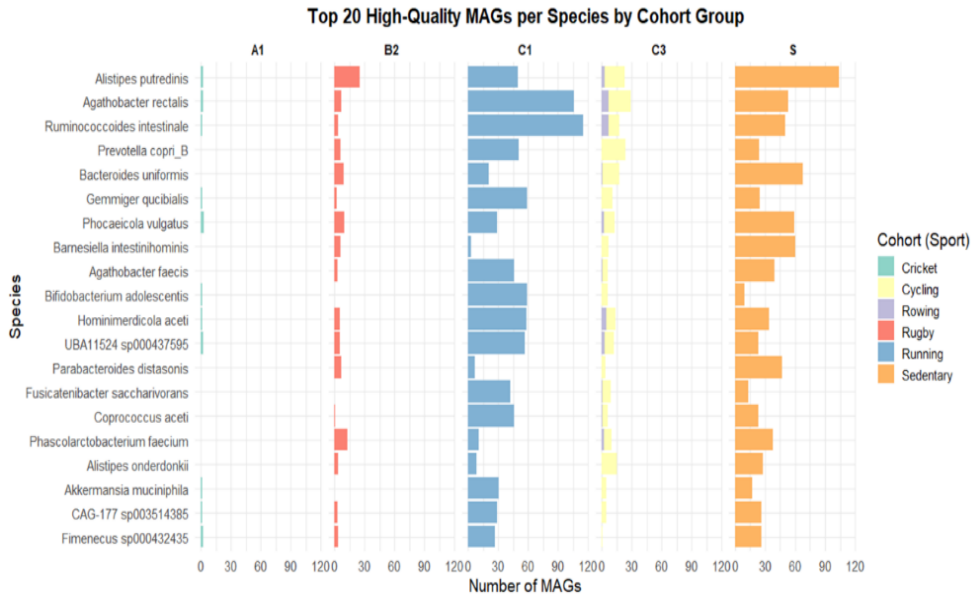


Figure 67. Distribution of high quality MAGS per species by cohort group

Then we compare the total number of metagenome-assembled genomes (MAGs) per species

obtained using two different binning methods: MetaBAT (red bars) and MetaWRAP (blue bars). The x-axis shows the species, and the y-axis represents the total number of MAGs identified for each species. A notable observation is that MetaWRAP (blue bars) generally identifies a higher number of high-quality (HQ) MAGs compared to MetaBAT (red bars), especially for species like *Alistipes putredinis*, *Agathobacter rectalis*, and *Ruminococcaceae intestinalis*, where MetaWRAP outperforms MetaBAT by a considerable margin (Figure 68). This suggests that MetaWRAP might be more effective in generating high-quality MAGs across a wider range of species. On the other hand, certain species like *Fusicatobacterium saccharivorans* show relatively similar numbers of HQ MAGs between the two methods. The differences in the number of HQ MAGs between MetaBAT and MetaWRAP highlight the variability in binning method performance and the potential of MetaWRAP for identifying more reliable MAGs for downstream analyses.

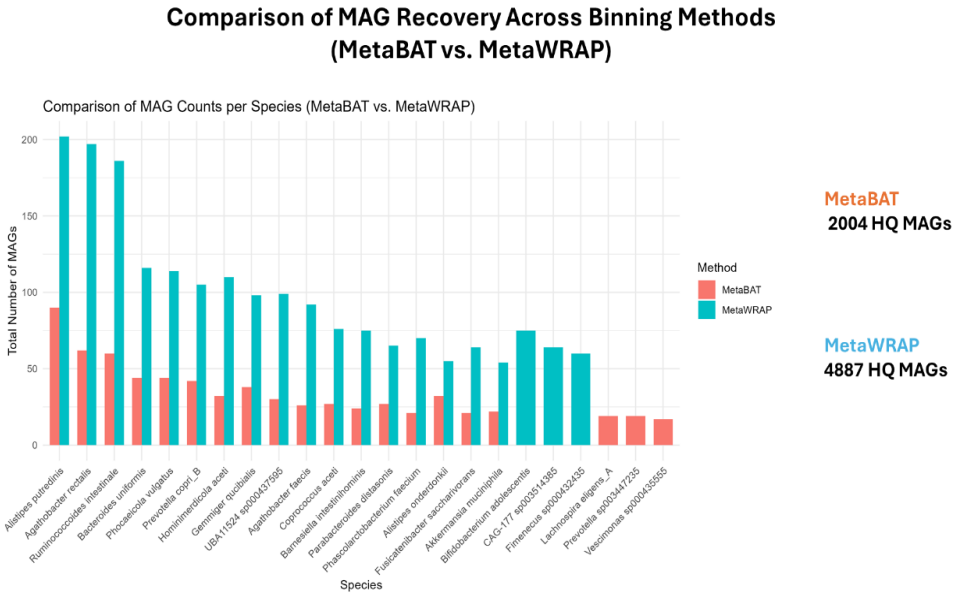


Figure 68. Comparison of MAGS per species across two binning tools

5.5.3.2 MAGs-based taxonomic profiling across sports classification group groups

Taxonomic assignment of HQ MAGs using GTDB-based classification showed broad representation across bacterial lineages. HQ MAGs were distributed across multiple phyla, with dominant contributions from taxa typically abundant in the human gut microbiome. The distribution of HQ MAGs across species and exercise-intensity groups suggested differences in genome-level representation between high-intensity athletes and sedentary controls, consistent with the diversity and clustering patterns observed at the community level. Heatmap-based visualization of taxonomic composition as shown in figure 69 revealed clear differences

in the relative abundance patterns of dominant gut microbial taxa across exercise-intensity groups. From the heatmap, it is evident that certain species such as *Prevotella copri B*, *Alistipes putredinis*, and *Bacteroides uniformis* have a high presence across specific groups (for example, *Prevotella copri B* is most prevalent in the A1 group). Other species, like *Phocaeicola vulgatus* and *Hominimerdicola aceti*, exhibit varying levels of presence across groups. The clustering of species and the varying presence/absence patterns across groups provide insights into how microbial diversity is influenced by different activity levels or sedentary behavior. This analysis helps highlight group-specific microbial signatures and differences in species composition across the study cohorts. These heatmap-based patterns are consistent with the diversity and ordination analyses, reinforcing the presence of exercise-intensity–related differences in gut microbiome organization.

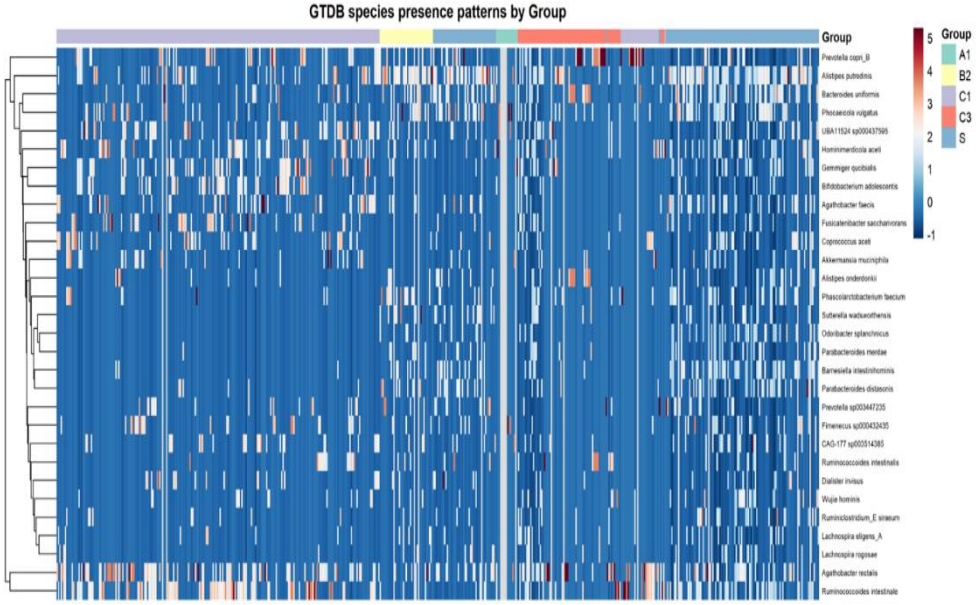


Figure 69. Heatmap of species patterns based on GTDB taxonomy in sports classification groups

Further analysis of species presence/absence across the exercise-intensity groups provided deeper insights into microbial diversity. The heatmap visualization of species as shown in figure 70 presence patterns across the groups (A1, B2, C1, C3, and S) revealed distinct differences in species composition, particularly between high-intensity athletes and sedentary controls. While certain species, such as *Agathobacter rectalis*, *Ruminococcaceae intestinalis*, and *Alistipes putredinis*, were widely distributed across all groups, others showed more pronounced presence in specific cohorts. For instance, *Prevotella copri B* and *Phocaeicola vulgatus* were notably more prevalent in group A1 (cricket), while *Fusicatobacterium saccharivorans* and *Hominimerdicola aceti* were more commonly detected in group C1 (rowing). The presence/absence patterns also suggested that sedentary individuals (S) exhibited

a distinct microbiome, with certain species absent or present at lower frequencies compared to active groups. The absence of species like *Bacteroides uniformis* in the sedentary group further supports the hypothesis that exercise intensity might shape the gut microbiome composition by promoting certain microbial species associated with specific metabolic and immune functions.

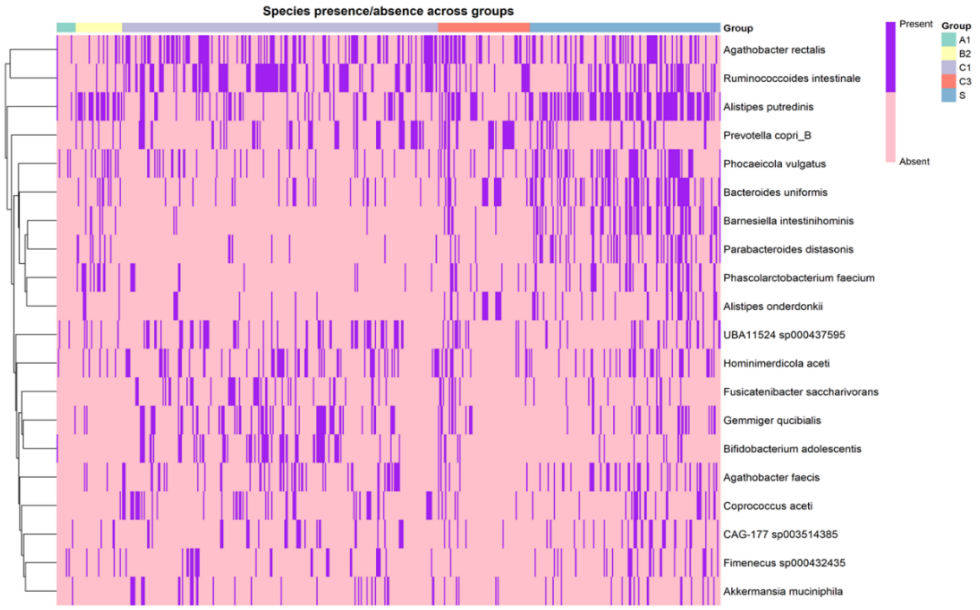


Figure 70. Heatmap of species presence/abscence based on GTDB taxonomy in sports classification groups

The dendrogram alongside the heatmap highlights clustering patterns of species that share similar presence profiles across the cohorts, suggesting that certain species may be specifically linked to higher physical activity levels or sedentary behaviours. These findings are consistent with earlier diversity and clustering analyses, reinforcing the notion that exercise intensity contributes significantly to shaping the gut microbiome's structure and species distribution.

6 DISCUSSIONS

Research on the gut microbiota is increasingly reshaping how health, disease, and physiological adaptation are understood, and it is becoming particularly relevant in sports science, preventive medicine, and lifestyle-based interventions. The gut microbiota contributes to metabolism, immune regulation, intestinal barrier function, and gut–brain communication, meaning that it may play a meaningful role in how individuals respond to exercise, stress, diet, and recovery-related behaviours. However, despite growing interest in the exercise–microbiota relationship, much of the existing literature has focused on elite athletes, vigorous training loads, and performance-oriented cohorts, leaving a substantial gap in relation to sustainable, non-

competitive, and mind–body forms of movement. This study addresses this gap by conducting the systematic reviews and meta-analysis to examine how exercise type and intensity, nutritional context, sex-related biological variation, and meditative engagement may together shape microbiota profiles and influence their interpretation. This broader perspective is important because it suggests that microbial responses to movement are not explained by physical activity alone but instead emerge from a more complex interaction of physiological, behavioural, and contextual factors. Overall, this study supports the view that the relationship between exercise and the gut microbiota cannot be reduced to a simple distinction between active and sedentary individuals. Rather, the combined findings suggest that microbial variation is influenced by the intensity and quality of exercise, by lifestyle and recovery context, and by host-level factors such as sex and meditative or psychological engagement.

The systematic review and meta-analysis conducted in this study reveal that both exercise intensity and intervention type influenced the *Firmicutes/Bacteroidetes* ratio, while at the same time highlighting the large heterogeneity of the available literature. These findings align with the broader conclusion of the review that different physical activity patterns produce different microbial responses and that no single exercise exposure can be assumed to have a uniform effect across populations. In this respect, this study is consistent with earlier studies summarized in the review, such as Moitinho-Silva et al., who reported distinct microbial modifications associated with endurance and strength exercise (Moitinho-Silva et al., 2021), and with Liang et al., who observed increased diversity and higher metabolic capacity in martial artists performing high-level exercise compared with those practicing lower-level exercise (Liang, Zhang, Peng, et al., 2019). This study therefore extends this line of reasoning by showing that even within movement disciplines that are not conventionally classified as high-intensity sport, important microbial differences may still emerge according to exercise structure and meditative context.

At the same time, the review component of the thesis makes it clear that exercise effects should not be interpreted in isolation from other variables. This is evident from the heterogeneity observed across the included studies, where differences in diet, supplementation, training duration, participant characteristics, and methodological design complicate direct comparison. These observations are in line with the study by Karl et al., also discussed in this study, which showed that physiological stress during military training was associated with altered intestinal permeability and microbiota composition (Karl et al., 2017), and with Jang et al., whose work suggested that diet quality may substantially modify the microbial effects seen in athletes (Jang et al., 2019). Similarly, the study cites Kulecka et al., who demonstrated that even a single

exercise bout can induce significant changes in microbiota and metabolomic markers (Kulecka et al., 2020), and Barton et al., who showed that sustained aerobic and resistance exercise over six months altered gut microbiota and physiologically relevant metabolites (Barton et al., 2021). Taken together, these findings reinforce an important interpretive point for the current study: exercise-related microbial variation is likely to reflect not only training load, but also the nutritional and physiological environment in which exercise takes place. This may explain why the literature often appears inconsistent and why the current study places such emphasis on context rather than on exercise alone as the explanatory variable.

The Aikido study included in this study provides a particularly important contribution because it suggests that meditative engagement may be more strongly associated with microbial variation than simple practice frequency alone. In that study, changes in gut microbiota were more evident across meditation-depth categories than across Aikido practice levels, with *Bacteroides*, *Faecalibacterium*, and *Prevotella* emerging as dominant genera and with stronger fluctuations reported for meditation groups. These findings are consistent with the rationale developed in the study itself, which argues that meditation may influence gut function through stress reduction, improved barrier function, and reduced inflammation. They also align with previous findings, including observations from long-term meditation practitioners in whom genera such as *Prevotella* and *Faecalibacterium* were enriched in deeper meditative states. In this sense, the present findings are not isolated but fit within an emerging body of work suggesting that mindfulness-related practices may influence the gut–brain axis and, through that route, potentially shape microbial ecology.

The comparative Tai Chi and Aikido study strengthens this interpretation by showing that mind body disciplines should not be treated as interchangeable exposures. Although alpha diversity remained broadly similar across groups, beta-diversity analyses and genus-level patterns suggested that the two practices were associated with distinct microbial community structures. Aikido was associated with higher relative abundance of genera such as *Faecalibacterium* and *Roseburia*, whereas Tai Chi showed relatively higher abundance of *Prevotella* and *Collinsella*. These findings are consistent with the study's own discussion, which notes that qualitative characteristics of movement may influence microbial communities independently of conventional exercise descriptors such as duration or volume. They also support the suggestion that movement style, breath control, and mind body synchronization may contribute to gut microbiota variation, even when the overall exercise intensity is relatively moderate. In contrast to more traditional athlete studies, where training load is often assumed to be the dominant variable, the present findings imply that movement quality and psychophysiological regulation

may also be relevant determinants of gut microbial composition. This is an important thesis-level contribution because it expands the field beyond the assumption that only vigorous or competitive exercise has meaningful microbiota consequences.

Another major strength of this study is that it does not interpret exercise and microbiota associations without considering the broader nutritional and recovery context in which physical activity occurs. This is where the Ramadan intermittent fasting review becomes particularly valuable. Although that review addressed athletic performance rather than the microbiota directly, it showed that the effects of fasting are strongly influenced by sport category, carbohydrate intake, training duration, hydration, sleep time, and recovery methods, with more negative effects often observed in team sports than in individual or combat sports. These findings align with the thesis-wide argument that behavioural context matters, because the same variables that influence performance during fasting such as sleep disruption, altered meal timing, hormonal changes, and dehydration are also plausible modifiers of gut microbial ecology. In this respect, the fasting review helps explain why microbiota patterns in active populations are difficult to interpret if nutrition timing, sleep quality, and recovery behaviour are not measured carefully. It also strengthens this study methodologically, because it demonstrates that exercise-related physiology should be understood as part of a wider adaptive system rather than as an isolated training effect.

A similarly important interpretive contribution comes from the review on sex-related differences in gut microbiota. The evidence summarized in the thesis suggests that sex is a relevant but not dominant contributor to microbial variability, with some studies reporting higher abundance of taxa such as *Akkermansia* and *Bifidobacterium* in females, and others showing stronger *Prevotella*- or *Escherichia*-related patterns in males. However, the review also makes clear that these differences are highly context dependent and may vary according to age, geography, hormonal status, diet, and methodological approach. This interpretation is consistent with the Tai Chi and Aikido cohort results presented in the thesis, where sex-related clustering was modest and appeared less influential than practice-related or meditation-related patterns. Thus, rather than treating sex as the primary explanatory variable, the present study supports a more balanced conclusion: sex should be considered as a biological factor and adjusted for analytically, but its effects are best understood as interacting with other variables rather than overriding them. This is important because it prevents overinterpretation of sex differences while still recognizing the biological nuance they introduce.

The oral microbiota analyses in Tai Chi practitioners add an important second body site to this stuy and broaden the interpretation beyond the gut microbiota alone. The oral profiles were

dominated by Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteriota at the phylum level and by Prevotella, Neisseria, Streptococcus, and Veillonella at the genus level, which is broadly consistent with a typical oral ecosystem. As in the analysis, alpha diversity did not differ significantly by meditation depth, sport level, sex, or BMI. However, the PCoA plots suggested structured differences in beta diversity across both meditation and sport levels. This pattern again supports the idea that lifestyle and practice variables may be associated more strongly with community composition than with overall richness.

The salivary biomarker findings help place these compositional patterns into a physiological context. Among the three biomarkers tested, MMP9 was the most responsive, with lower concentrations in participants at higher sport levels. Because MMP9 is commonly interpreted as an indicator of inflammatory and tissue-remodeling activity, this result may reflect an adaptive reduction in inflammatory tone among individuals with higher habitual activity (Sielczak et al., 2025). In practical terms, it suggests that regular Tai Chi practice performed at a higher activity level may be associated with a less inflammatory oral environment. By contrast, S100B showed high within-group variability and no significant associations, while GFAP was below the lower limit of quantification in most samples and therefore could not provide strong inferential value. These null findings are still useful because they indicate that not all salivary biomarkers are equally informative in healthy community-dwelling practitioners (Kim et al., 2025). From a mechanistic perspective, the oral findings are important because they suggest that contemplative practice may be reflected in both microbial and host inflammatory signals, albeit more clearly for sport level than for meditation depth. The pattern is also consistent with the broader study argument that physical activity and mind-body practices may influence host-microbe systems through overlapping but non-identical pathways. Oral ecology is sensitive to hydration, breathing pattern, diet, hygiene practices, and mucosal immunity, so it is plausible that Tai Chi-related changes in breathing regulation, stress response, or exercise dose could shape the oral environment in ways that are not fully mirrored in standard alpha-diversity indices

Further the metagenomic component provided the clearest evidence in this study for an exercise-intensity gradient in gut microbiota organization. Across the publicly available athlete cohorts, high-intensity groups exhibited the highest alpha diversity, whereas sedentary controls consistently showed the lowest diversity and the greatest between-subject dispersion. Community-level ordinations and PERMANOVA results further showed that high-intensity athletes were more clearly separated from sedentary controls than athlete groups were from each other. This suggests that sustained training load is associated not only with richer

microbial communities but also with more coherent ecological structuring (O'Donovan et al., 2020). In this respect, the metagenomic analysis aligns closely with the conducted systematic review and meta-analysis, which also indicated that exercise intensity is an important determinant of microbiota composition and of the *Firmicutes/Bacteroidetes* ratio.

The species patterns identified in the metagenomic analysis further strengthen this interpretation. High-intensity groups were characterized by greater representation of organisms such as *Faecalibacterium prausnitzii, Roseburia intestinalis*, and *Bacteroides uniformis*, all of which are frequently discussed in relation to short-chain fatty acid production, mucosal health, and anti-inflammatory metabolism (Barton et al., 2018; Palmnäs-Bédard et al., 2022). In contrast, sedentary controls showed a more dispersed and variable microbiota, which may reflect greater heterogeneity in diet, lifestyle, and metabolic status. The genome-resolved approach also adds methodological depth to the thesis because it moves beyond amplicon-based relative abundance and demonstrates that group differences are also visible at the level of reconstructed microbial genomes.

At the same time, this component of the study also illustrates the complexity of interpreting athlete microbiomes. The exercise categories were constructed by harmonizing multiple cohorts that differed in sport type, country, sequencing context, and diet. Therefore, exercise intensity cannot be viewed as the sole driver of all observed differences. Some of the signals attributed to intensity may partly capture sport-specific routines, nutritional strategies, travel, or other cohort effects. Even so, the consistency of the diversity gradient from sedentary controls to high-intensity athletes, together with the presence of distinct species signatures and lower beta-dispersion in the more active groups, supports the conclusion that exercise intensity is a major organizing factor in the athletic gut microbiome.

When considered together, the studies support a multidimensional interpretation of microbiota modulation in physically active populations. The systematic review suggests that exercise intensity and intervention type matter; the Aikido and Tai Chi studies indicate that meditative engagement and movement style may further shape community composition; the fasting review demonstrates that nutrition timing, hydration, and sleep strongly modify physiological responses to exercise; and the sex-differences review adds an additional layer of biological variation. These findings align with contemporary views of the gut–brain–muscle axis and support the argument that microbial responses to exercise are not simply mechanical consequences of training load, but reflect the interaction of metabolism, stress regulation, recovery, and behaviour. This may also explain why certain genera highlighted in the current

study, such as *Faecalibacterium* and *Roseburia*, are often discussed in relation to beneficial metabolic potential, while taxa such as *Prevotella* appear to have more context-dependent interpretations depending on the wider microbial and dietary environment. Importantly, however, the current thesis also recognizes that such interpretations remain inferential in the absence of direct functional or metabolomic measurements. Despite these promising findings, several limitations should be acknowledged. First, the original gut microbiota studies in the thesis are exploratory and cross-sectional, which means that they cannot establish causality and remain vulnerable to residual confounding from unmeasured variables such as dietary composition, stress outside training, sleep patterns, and broader lifestyle routines. Second, subgroup sizes were relatively modest, and some meditation-level strata were particularly small, which limits statistical power and means that interaction signals should be interpreted cautiously. Third, the use of 16S rRNA sequencing provides valuable compositional information but limited functional resolution, which makes it difficult to determine whether observed taxonomic patterns correspond to meaningful metabolic differences. These limitations are also acknowledged in the Tai Chi/Aikido paper itself, which explicitly describes its findings as associative and hypothesis-generating rather than causal. Nevertheless, these limitations do not weaken the value of the thesis; instead, they define its role as an important exploratory framework that identifies biologically plausible patterns and justifies more rigorous future work. At the same time, this study has several notable strengths. It integrates evidence across multiple study designs, combining a systematic review and meta-analysis with original observational work in understudied meditative movement populations. It also strengthens interpretation by explicitly incorporating reviews on fasting and sex differences, thereby acknowledging two major sources of variability that are often overlooked in microbiome exercise research. In addition, the thesis broadens the field conceptually by moving beyond elite or high-performance models and focusing on movement practices that are accessible, sustainable, and relevant to the general population. This is a meaningful contribution because practices such as Aikido and Tai Chi may provide valuable models for studying how physical activity, stress regulation, and body awareness interact to shape host–microbe relationships in everyday life, rather than only in elite sport environments.

From a practical and scientific perspective, the integrated findings of this study suggest that future microbiome research in sport and movement science should move toward more precise and context-aware designs. Future studies should distinguish clearly between exercise modalities rather than grouping them under broad labels, should measure diet timing, hydration, sleep, and recovery behaviours more systematically, and should include balanced

sex-stratified analyses where feasible. Longitudinal and intervention-based designs will be especially important for determining whether observed differences in meditative movement cohorts represent stable training-related effects or transient associations shaped by lifestyle. In addition, multiomics approaches, including metagenomics and metabolomics, will be necessary to determine whether the taxonomic differences observed in the present thesis correspond to meaningful functional consequences. Such developments would help move the field from descriptive microbiota profiling toward a more mechanistic understanding of how active lifestyles influence host–microbial interactions. This study provides a strong argument that microbiota variation in physically active individuals is shaped by more than exercise volume or intensity alone. The integrated findings indicate that movement modality, meditative engagement, nutritional timing, sleep and recovery context, and sex-related biological factors all contribute to the wider microbial landscape. By combining evidence from systematic reviews with original studies in Aikido and Tai Chi practitioners, the thesis expands the exercise–microbiota field beyond narrow athlete-centred models and introduces meditative movement as a meaningful and understudied research context. In doing so, it contributes to a more sophisticated understanding of how physical activity, behaviour, and physiology converge in shaping host microbe interactions that may be relevant to health, adaptation, and performance.

7 CONCLUSIONS AND PERSPECTIVES

This thesis sets out to examine the relationship between sport, contemplative movement, and the microbiota, with particular emphasis on Tai Chi and Aikido as mind-body practices. Across its different components, the thesis shows that the microbiota is meaningfully associated with physical activity, but that the nature of this association depends on the type of movement under consideration. The systematic review and meta-analysis indicated that exercise intensity is an important determinant of gut microbial organization, including changes in the *Firmicutes/Bacteroidetes* ratio. The metagenomic comparison of athlete cohorts reinforced this conclusion by showing that high-intensity athletes tend to display higher alpha diversity, clearer separation in beta diversity, and more coherent species-level patterns than sedentary controls. These findings support the view that sustained physical training exerts a measurable effect on gut microbial ecology.

At the same time, the original studies on Tai Chi and Aikido suggest that contemplative

movement practices should not be interpreted through intensity alone. In the Aikido cohort, deeper meditation was associated with a reduction in Shannon diversity but also with taxonomic shifts that may reflect a more selective and potentially favorable microbial configuration, including higher representation of *Bacteroides* and *Faecalibacterium*. In the combined Tai Chi and Aikido cohort, meditation level and the interaction between practice type and meditation depth were more informative for beta diversity than for alpha diversity, indicating that meditative engagement may be associated primarily with community restructuring rather than with simple increases or decreases in richness. Moreover, Tai Chi and Aikido were associated with different microbial signatures, suggesting that distinct contemplative movement disciplines may shape the gut in different ways. The oral microbiota findings extend this interpretation beyond the gut and show that the relationship between meditative movement and host-microbe biology may involve multiple body sites. Although alpha diversity in the oral microbiome did not differ significantly across groups, beta-diversity patterns suggested that both meditation depth and sport level may influence oral microbial structure. In addition, salivary MMP9 concentrations were lower in the higher sport-level group, pointing toward a possible link between sustained practice and reduced inflammatory tone. By contrast, S100B and GFAP were not clearly informative in this healthy cohort.

Taking together, these results suggest that the biological relevance of contemplative movement may be detectable not only in microbial composition but also in selected host biomarkers.

The main conclusion of the thesis is therefore that exercise-microbiome relationships should be understood along at least two complementary dimensions. The first is physical intensity, which strongly shapes microbial diversity and community structure in broad athlete populations. The second is psychophysiological quality, including meditation depth, attentional focus, and stress regulation, which may shape microbial composition in more subtle but still meaningful ways within contemplative practices. This perspective is important because it widens the conceptual scope of microbiome research beyond elite sport and highlights the potential value of accessible movement disciplines such as Tai Chi and Aikido.

From a practical and scientific point of view, the thesis also raises important methodological considerations for future work. A significant limitation of the present study is the absence of dietary assessment data. Diet is widely recognised as the single most important modifiable determinant of gut microbiome composition, with macronutrient content, dietary fibre intake, and protein sources each independently associated with shifts in microbial community structure. Tai Chi and Aikido practitioners may differ systematically in habitual dietary patterns i.e. in fibre intake, plant-based food consumption, or protein sources in ways that could

independently account for the microbial differences observed between groups. Without concurrent dietary records, the relative contribution of contemplative practice cannot be fully disentangled from that of habitual diet. Larger controlled studies are needed to verify whether the compositional differences observed in Aikido and Tai Chi remain stable after adjustment for diet, sex, age, training history, and other lifestyle factors. Moreover the Aikido and Tai Chi cohorts were not recruited concurrently Aikido data were drawn from Aikido Study and collected at an earlier time point, while Tai Chi participants were recruited de novo for this study. This temporal separation represents a cohort-level confound that cannot be fully controlled for analytically, as it introduces the possibility of sequencing batch variation and seasonal fluctuations in gut microbial composition; between-group differences should therefore be interpreted with this caveat in mind. Longitudinal interventions would be particularly valuable for determining whether beginning or intensifying a contemplative movement practice can directly modify the microbiota over time. Beyond their scientific value, such interventions would have direct relevance for practitioners and coaches working within contemplative martial arts communities. If specific microbial signatures can be linked to training outcomes, meditation depth, or inflammatory tone as preliminary evidence here suggests it would become possible to use microbiome profiling as a non-invasive monitoring tool in athletic populations who prioritise mind-body regulation alongside physical performance. In disciplines such as Tai Chi and Aikido, where sustained practice is associated with measurable shifts in oral and gut microbial structure, routine microbiome assessment could complement existing measures of athletic development and health.

This study has several notable methodological and conceptual strengths. From a methodological standpoint, participants with recent antibiotic use were excluded prior to enrolment, removing one of the most potent short-term confounders of gut microbial composition. Identical primers were used and sequencing depth was equalised across all samples, ensuring that compositional comparisons were not confounded by differential amplification efficiency or unequal library sizes. Both cohorts were collected under the same ethical protocol and within the same geographic setting, which improves the internal comparability of the groups and reduces broad dietary and cultural heterogeneity as a source of between-group variation. Conceptually, the thesis integrates evidence across multiple study designs combining a systematic review and meta-analysis with original observational work in two understudied meditative movement populations providing a more comprehensive interpretation than any single study design could offer in isolation. The thesis also strengthens its interpretive framework by explicitly incorporating reviews on nutritional context and sex-

related biological variation, thereby acknowledging two major sources of variability that are frequently overlooked in exercise-microbiome research. Finally, by focusing on Tai Chi and Aikido rather than elite or high-performance sport, this study broadens the conceptual scope of the field and highlights movement practices that are accessible, sustainable, and relevant to the general population an important contribution given the growing public health interest in lifestyle-based strategies for supporting gastrointestinal and metabolic health.

Future research should also integrate functional tools such as shotgun metagenomics, metabolomics, short-chain fatty acid profiling, and inflammatory or autonomic markers to test the gut-brain-muscle hypothesis more directly. In conclusion, the present thesis contributes to an emerging field at the intersection of microbiology, exercise physiology, and mind-body medicine. Its findings support the idea that microbiota is responsive to human movement across a broad continuum, from high-intensity sport to contemplative martial arts. More importantly, it suggests that the biological meaning of exercise does not depend only on workload, but also on the way movement is embodied, regulated, and repeated over time. Recognizing this broader framework may help shape future lifestyle-based strategies for promoting gastrointestinal, metabolic, oral, and psychological health.

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Systematic Review

The Role of Gut Microbiota in Different Types of Physical Activity and Their Intensity: Systematic Review and Meta-Analysis

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Abstract: Background. Intense exercise during training requires dietary modulation to support health and performance and differs in different types of activities. Diet, supplementation with prebiotics and probiotics, and, more recently, even physical activity can potentially improve health outcomes by modifying and protecting the gut microbiota. A systematic review and meta-analysis were conducted to investigate the modulation of gut microbiota in different types and intensities of physical activity and different lifestyles of athletes. Methods. The systematic review and meta-analysis were conducted according to the PRISMA guidelines, and the protocol was registered in PROSPERO (CRD42024500826). Results. Out of 1318 studies, only 10 met the criteria for inclusion in the meta-analysis. The pilot study's meta-regression analysis highlights the role of type and intensity of exercise in changing the B/B (Bacillota/Bacteroidota) ratio ($p = 0.001$). Conclusions. As gut training becomes more popular among athletes, it is necessary to map interactions between microbiota and different types of physical activity, personalized diets, physical activities, and ergogenic supplements to enhance performance and athletic wellness.

Keywords: gut microbiota; physical activity; physiological factors; diet; athletes

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1. Introduction

The human gut microbiota is the population of microbes, including bacteria, archaea, eukaryotes, and viruses that inhabit our gastrointestinal tract [1]. It has coevolved with humankind and is thought to number approximately 100 trillion [1,2]. The main bacteria taxa are in different relative amounts, such as Bacillota, Bacteroidota, Cyanobacteria, Pseudomonadota, Fusobacteria, Actinomycetota, and Verrucomicrobiota. The first two phyla represent about 90% of the bacterial flora population in the gut, especially the colon [3,4]. Gut microbiotas possess metabolic, protective, and structural functions in the gut mucosa [5]. Over the years of research and development of sequencing technologies and projects such as the Human Microbiome Project (HMP), the knowledge of the human microbiome has increased as well as the awareness about the host–microbial flora interaction influencing the health status of humans [6]. The gut microbiota matures during the first years of life, but its development depends upon several factors like the mode of birth, dietary interventions, intake of antibiotics, and the interference of some environmental factors including exercise, diet, and stress that can lead to a state of eubiosis or dysbiosis [7].

Scientific evidence shows that these groups of organisms are not only involved in local processes such as maintaining the balance of the mucous membranes and preserving the integrity of the epithelial cells, as well as in the production and absorption of nutrients, but they also play a role in the immune system and the nervous system, forming part of the brain–gut axis [4,8]. In particular, gut microbes can change the homeostasis of the

host by producing vitamins, short-chain fatty acids, and amino acids starting from food components [9,10]. Sport and physical activity, including leisure activities, planned exercise, and various sports disciplines, are essential for maintaining a healthy lifestyle and are fundamental tools in preventing and treating many chronic diseases by influencing the intestinal microbiota [11].

Research shows that certain groups of microorganisms, such as SCFA producers, are associated with exercise. These microorganisms may play a role in maintaining the balance of the intestinal lining, increasing mucus thickness, improving the body's metabolic and immune functions, and influencing the connection between the gut and the brain. As a result, they may help reduce neuroinflammation and mental fatigue [12–14]. Exercise intensity determines the effect of gut microbial diversity as low-intensity exercise reduces the transit time of stool and ultimately reduces the connection time between the microbiota and mucus layer. Endurance exercises lead to changes in the gastrointestinal tract, leading to reduced splanchnic blood flow to almost 80% of the basal level [15]. Prolonged exercises possess beneficial effects like bacterial translocation from the colon, increased intestinal permeability, and a compromised gut barrier [5].

The intensity of the physical activity modulates the gut microbiota and a number of short-chain fatty acids. Aerobic exercises performed for at least 60 min and 60% HRmax per day tremendously increased the beta diversity indexes of gut microbiota and SCFAs [16]. The gut microbiota is influenced by the physical activity and the dietary intake of athletes, collectively influencing physical performance, so boosting the athletes' capabilities is essential to fuel the gut bacteria through diet and exercise. Especially high fiber diets, protein-rich foods, unsaturated fats, and supplements have a positive impact [17]. The endurance exercise impact gut bacteria depending on the subject and its intensity. It was found that in athletes, endurance exercises depict a negative effect on the intensity of gut bacteria, while in non-athletes, this effect was less as compared to athletes [18].

Exercise-related stress might be associated with changes in the structure of the intestinal microbiota. The evaluation of associations among gut microbiota and diet and exercise in different athletes such as bodybuilders and elite runners was considered. The results indicate differences in gut microbiota diversity according to athlete, sport type, and diet [19]. Also, experimental studies on animal models have accessed the interaction between gut microbiota and exercise intensity. The stool samples of animal models collected at different intervals were analyzed using 16S amplicon sequencing, and moderate-intensity exercise seems to affect both the gut microbiota and metabolism [20]. An observational trial recruiting elite athletes performing endurance training and consuming high protein and high carbohydrate diets showed how the dietary intake may impact gut flora with implications on the performance [21].

The athlete's gut microbiota differs significantly from non-athletes in terms of both composition and metabolic functioning [22]. Physical activity is linked with a tremendous increase in gut microbial diversity and an increment in the yield of short-chain fatty acids by bacteria which leads to an increase in butyrate production. The SCFAs have a role in maintaining homeostasis, epithelial integrity, mental fatigue, and neuroinflammation.

Scientific evidence differs in several issues, such as the kind of biodiversity change and the role of dietary behavior vs. physical activity or other variables [13,23,24]. A systematic review conducted in 2021 shows that most of the included papers registered greater variability and frequency of the phylum Bacillota in active versus sedentary subjects. Furthermore, studies that have performed dietary control have reported associations between physical activity and some bacterial families, including Lachnospiraceae, Clostridiaceae, Paraprevotellaceae, Ruminococcaceae, and Veillonellaceae [23]. An experimental study conducted on young Caucasian adults also showed a positive correlation of the phylum Bacillota with BMI [24]. However, it is necessary to further investigate how specific foods or supplements can modulate the possible influence of exercise on microbial diversity in the gut [13].

Furthermore, human studies, at least to date, have a heterogeneous model design, approaching different kinds, doses, and durations of exercise [9]. Experimental studies revealed a strong relationship between microbiota, diet, sport, health, and athletic performance [25]. Given the vast diversity and number of gut microbiota and their interaction with exercise, the data may not always be current or authentic, making the literature on the role of microbiota seem scarce [25–28]. Nonetheless, existing data predicts that the microbiome can be influenced positively or negatively, depending on specific dietary intake, type, intensity, and duration of exercise. This implies that a deeper investigation into the role of individual species could lead to strategies designed to promote beneficial gut microbiota and suppress harmful ones. Therefore, it is crucial to expand our understanding of how gut microbiota interact with various types of exercise [28]. This review seeks to examine how different types and intensities of physical activity affect microbiota diversity.

2. Materials and Methods

2.1. Selection Protocol and Search Strategy

The present review was conducted according to the PRISMA guidelines [29]. The protocol was registered in PROSPERO (Reference Number CRD42024500826). PICOS frame and the specific eligibility criteria we used to perform this review. Especially, the criteria are (a) population health subjects; (b) intervention of any type of physical activity; (c) comparison not physical conditioning or other types of physical training; (d) outgrowth assessment of differences for α-diversity and β-diversity, relative diversity of specific bacteria, and/or metagenomic data anatomized with 16S amplicon sequencing; (e) papers written in English; (f) observational studies (cross-sectional, retrospective, and prospective studies) and experimental study design. Clinical trials, reviews, meta-analyses, case studies, case reports, proceedings, qualitative studies, commentary studies, and studies without a control group, as well as studies with unhealthy subjects and incomplete designs, were excluded. PubMed, ScienceDirect, and Scopus databases were accessed using the following terms ("gut microbiota, physical activity" [MeSH Terms] OR ("microbiota" [All Fields] AND "physical activity" [All Fields] AND "diet" [All Fields]) OR "human gut microbiota" [All Fields] OR ("microbiota" [All Fields] AND "Gut" [All Fields] AND "physical activity" [All Fields])) AND ("microbiota" [MeSH Terms] OR "microbiota" [All Fields] OR "microbiotas" [All Fields] OR "microbiotas" [All Fields] OR "microbiota" [All Fields] OR "microbiome"). The search string used on PubMed was carried out by title, abstract, and MeSH terms; the hunt on Scopus and Web of Science involved content by the title, abstract, and keywords. The search was conducted until April 2023. The screening of studies consisted of a multistep rejection process, with four experts personally reviewing the titles and abstracts of the studies [29]. The titles and abstracts gathered from these three databases were then imported into covidence—a tool for better systematic review management—to facilitate the assessment of their relevance [30]. The coming step was screening by title and abstract the possibly eligible studies, following the additional criteria mentioned ahead; this step was performed by 4 authors (T.G., F.V., F.U., V.V.) singly. Also, full textbooks were read singly by the 4 authors (T.G., F.V., F.U., V.V.) with a discussion about their addition in the review. Dissensions were achieved by agreement among the authors.

2.2. Data Extraction Process and Quality Assessment

The data extracted from each eligible record included bibliographical information, design of the study, population characteristics, type of exercise, duration, frequency, training volume, and microbial profile analysis.

Adequate quality assessment tools for every type of study comprised in the review were used to assess the quality of eligible articles. Cross-sectional studies were calculated by the New Castle–Ottawa Quality adapted for cross-sectional studies. Cohort studies were assessed by the Newcastle–Ottawa Scale for Cohort Studies (NOS) [31–33]. A comprehensive quality rating was assigned to every eligible article depending on the number of values met as follows: good quality (all values met, low risk of bias); fair quality (1 value

not met or 2 values unclear, moderate risk of bias); poor quality (2 or more values not met, high risk of bias). Randomized clinical trials were evaluated by the Cochrane collaboration risk-of-prejudice tool for randomized trials (RoB2) [34]. Each RCT was graded according to five domains: randomization, deviation, missing data, outcome measurement, and selective reporting, and an overall score for risk of bias was assigned. Four reviewers (T.G., F.U., V.V., F.V.) independently assessed the risk of prejudice for the articles comprised, according to the four authors singly assigned a score to each study, and conflicts were settled by agreement among all the authors.

2.3. Data Synthesis

For differences in the overall content of the gut microbiota, we only performed a descriptive literature fusion because of the different situations of determination for the proportion of microbiota, the small sample sizes, and the insufficient data and quality of the involved studies. We also performed meta-analyses by using comprehensive meta-analysis [35]. The Bacillota/Bacteroidota (B/B) ratio, which is commonly used to assess gut health, was one of the indices considered in this study. In cases where there were issues with the unit of analysis, only the total number of participants in the control group were divided, and the means and standard deviations remained unchanged. The 95% confidence interval (95% CI) was also calculated. Effect size was distributed as small (SMD = 0.2), moderate (SMD = 0.5), and large (SMD = 0.8) [35]. The statistic test was used to assess the diversity of named studies and testing the classical measure of diversity is Cochran's Q (Hedges Q statistic). The thresholds test for the interpretation of I_2 were as follows: <25%, low diversity; <50%, moderate heterogeneity; and >75%, high diversity. To estimate the publication bias, due to the high volume of samples included, the Egger's test and channel plot were performed. Subgroup analyses were carried out for outcomes reported in studies with two or more groups within each subgroup. Meta-regression and subgroup analysis were used to find the expected sources of heterogeneity [36]. Predefined subgroup analyses were performed, including chronic or acute intervention, type of aerobic or anaerobic, intensity of physical activities, sample size, ages, publication year, and methodological quality of the study. For physical activity intensity, we used different classifications, based on a percentage of maximal aerobic power (VO_2 max and VO_2R) and metabolic equivalents (METs) values [37–39]. To perform a sensitivity analysis, we either excluded individual studies or modified the effects model [35,36,40].

3. Results

The identification of the specific physical activity is required to identify the strains of gut microbiota in the specimens. The purpose of determining the strains of bacterial species is to find the alterations in the gut biodiversity depending on the type and intensity of physical exercise [41,42]. In this context, the knowledge of microbiota structure and its modification is fundamental to drawing these interventions. Here, we aim to summarize the data on the microbiota diversity in athletes depending on their activity and how the different types of exercises can modify their composition.

Figure 1 shows the steps of the selection procedure for the systematic review [29,40]. In Table S1, the PRISMA checklist shows the location where the item is reported in the paper. In total, 1318 studies were retrieved from all searched databases and, after expelling duplicates, 127 articles remain for the following steps. Of the remaining studies, 100 were selected for full-text analysis. Then, the full texts of selected articles were accessed and evaluated, considering the inclusion and exclusion criteria. After the assessment, 81 articles were excluded based on the exclusion criteria. Finally, 19 articles fulfilled the inclusion criteria and were placed in the systematic review [42–60], and 10 articles were used for meta-analysis [43,46,50,51,53–56,58,59].

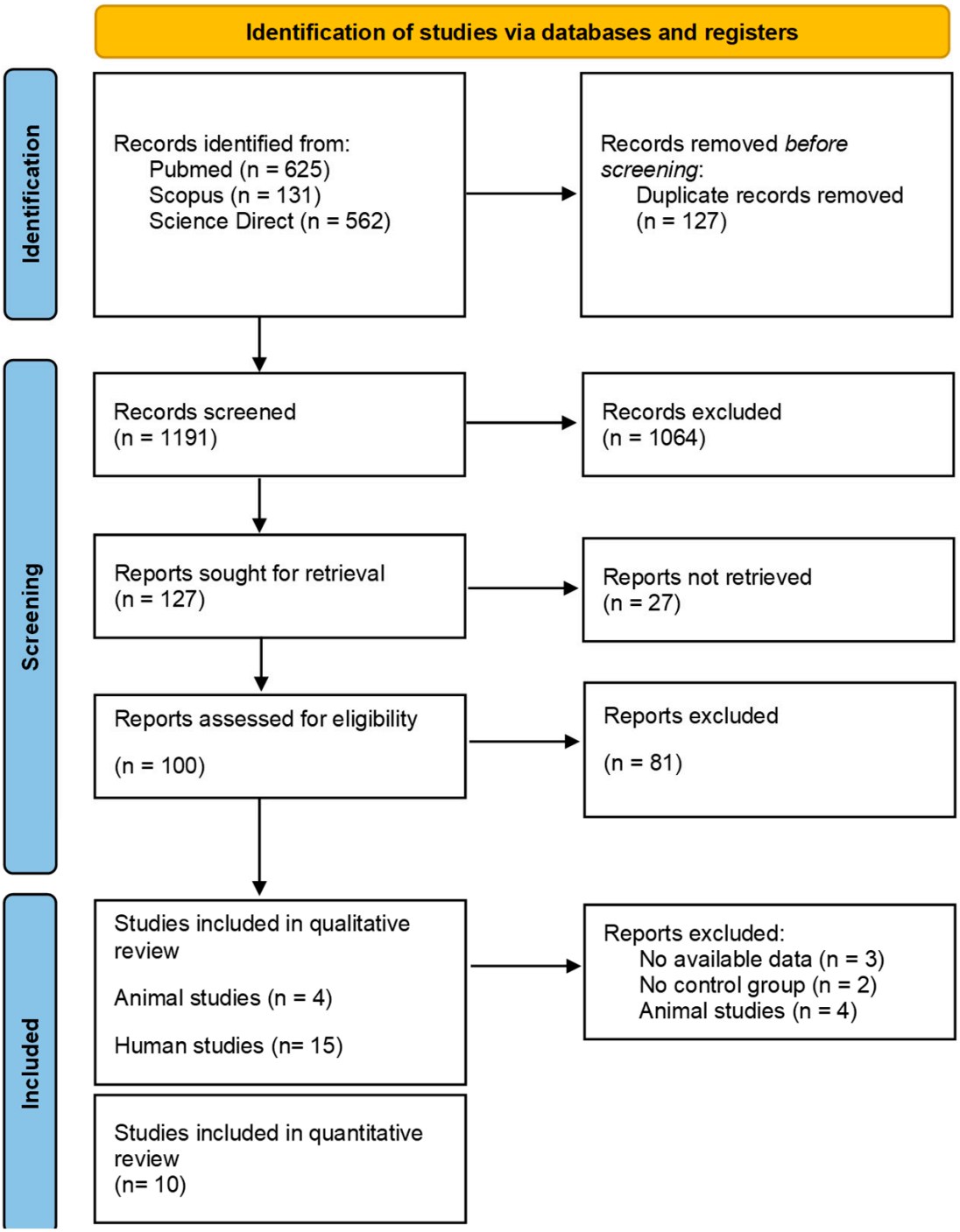


Figure 1. PRISMA flow diagram following the PRISMA guidelines [29,40].

Based on the results collected from the previously available narrative reviews, a meta-analysis was conducted, focusing on a few studies that could be covered in the systematic review. The studies enclosed in the systematic review are divided into two groups, one group contains all the animal studies (Table 1) [42,44–46], and the other group includes all the human studies (Table 2) [43,47–60].

Table 1. Characteristics of original studies and main results related to physical activity and microbiota (in animals). ND = Not described.

Author, Year, Country, Ref	Study Design	Subjects (Animals)	Sample Size	Age (Mean Value ± SD, Range/Age Group %)	Gender	Type of Sample	Type of Exercise	Duration, Frequency, Training Volume	Outcome	Quality Assessment (NOS Scale)
Pekkala, 2017, Finland [42]	Cohort study	Mice	20	7 weeks	Male and female	Fecal	Endurance exercise treadmill running	ND	Intrinsic aerobic capacity governs the microbiome, which may influence body weight, metabolism, and gene expression	8 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Yang, 2021, China [44]	Cohort study	Mice	30	5 weeks old	Male	Fecal	Moderate intensity treadmill exercise	3 days a week, 10–20 min a day, slope 0, speed 10–13 mile per minute	Moderate-intensity treadmill exercise significantly increased the exercise capacity of mice and alters the core bacteria and bacterial metabolic activities	6 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Mach, 2021, France [45]	Cohort study	Horse	20	10 ± 1.69 years	Male and female	Fecal, Blood	Endurance exercises	120 and 160 km race	Targeting the gut-mitochondria axis, therefore, appears to be a possible strategy to enhance athletic performance	6 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Tung, 2019, Taiwan [46]	Cohort study	Mice	100	7 weeks old	Male	Fecal	Exhaustive swimming	Exhaustive swimming test, with 5% body weight (BW) loading on the tail	The mice with various intrinsic exercise capacities have different gut microbiome as well as transcriptome and proteome of soleus muscle by applying multi-omics approaches. The main bacteria and controlling factors, including miRNA and functional proteins, may be too much correlated with the adoption of physiological functions and exercise capacity	6 (Newcastle–Ottawa Quality Assessment Scale) Cohort Studies

Table 2. Characteristics of original studies and main results related to physical activity and microbiota (in humans).

Author, Year, Country	Study Design	Sample Size	Age (Mean Value ± SD, Range/Age Group %)	Gender	Type of Exercise	Duration, Frequency, Training Volume	Outcome	Quality Assessment
Petersen, 2017, USA [43]	Cross-sectional study	33	19–49	Male and female	Cycling	All participants spent a minimum of 6 h exercising per week	No significant correlations were found between taxonomic cluster and being either a professional or amateur level cyclist, high abundance of the genus *Prevotella* (≥2.5%) was substantially correlated with time noted for exercising during an average week	5 (Newcastle–Ottawa Quality Assessment Scale) Adapted for cross-sectional studies
Karl, 2017, USA [47]	RCT	73	>18	Male and female	Fifty-one kilometer of cross-country ski-march	4 days artic military training (51-km cross-country ski-march, during which volunteers skied in the 50:10-min work-to-rest ratios also carrying a ~45-kg pack)	Physiological stress is associated with the intestinal permeability, changes in gut microbiota by modifying diet and stress level can change intestinal permeability	3 (Cochrane risk-of-bias tool for randomized trials)
Yang, 2017, China [48]	Cross-sectional study	71	19–49	Female	ND	2-min warm-up at 50 W to access VO_2 max	Cardiorespiratory fitness is associated with gut microbiota composition, independent of age and carbohydrate or fat intake	6 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies
Tabone,2021, France [49]	Cohort study	40	18–50	Male	Cross-country running	10-min warm-up of continuous running on a treadmill at 60% of their maximum heart rate. After the warm-up, they ran with a slope of 1% at a speed of 10 km/h, with increase of 0.3 km per hour every 30 s until volitional exhaustion	The performance of a single exercise bout in cross-country non-professional athletes produces significant changes in the microbiota, in serum and fecal metabolome, which may have health implications	8 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Oliveira, 2021, Portugal [50]	Cohort study	17	18–25	Female	Football	Perceived exertion method	The physical and physiological requirements of training and matches of an official international tournament did not vary the gut microbiota balance of elite female football players	6 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)

Table 2. *Cont.*

Author, Year, Country	Study Design	Sample Size	Age (Mean Value ± SD, Range/Age Group %)	Gender	Type of Exercise	Duration, Frequency, Training Volume	Outcome	Quality Assessment
Craven, 2021, Australia [51]	Cohort study	14	18–25	Male and female	Middle distance running	Three weeks of the normal training; 3 weeks of high-volume training (10, 20 and 30% increment in training volume during every successive week from NormTr); one-week taper (TaperTr; 55% exponential decrease in training volume from third HVolTr week)	The alpha-diversity and global formation of the gut microbiome were unaffected by differences in training volume. However, an increment in training volume led to various changes at the lower taxonomy levels that did not come back to pre-HVolTr levels following the taper period	8 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Moitinho-Silva, 2021, Germany [52]	RCT	42	20–45	Male and female	Strength exercise, endurance exercise	Control group (general physical activity), Endurance group (at least 30 min of running three times per week), and Strength group (three days per week of whole-body hypertrophy strength training in the gym). The participants warmed up for five minutes on the treadmill, ergometer, or rowing machine before beginning their 30-min training session. One session included six distinct exercises, two for each leg, chest, and back, to develop the big and main muscle groups. The participants did one warm-up set (which was supposed to be 50% of the load set weight) and one load set for each activity. The weight for the load set was chosen so that eight repetitions were possible. If more than eight repetitions are doable on two consecutive training days.	Various types of exercise have different but balanced effects on the overall physiology of humans and very versatile microbial changes in the gut	4 (Cochrane risk-of-bias tool for randomized trials)

Table 2. *Cont.*

Author, Year, Country	Study Design	Sample Size	Age (Mean Value ± SD, Range/Age Group %)	Gender	Type of Exercise	Duration, Frequency, Training Volume	Outcome	Quality Assessment
Han, 2020, China [53]	Cohort study	19	12–26	Female	Rowing	NA	Gut microbial communities depend upon the type of exercise associated with dietary factors and physical characteristics	7 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)
Liang, 2019, China [54]	Cross-sectional study	31	20–24	Male and female	Professional martial arts	Higher-level group: exercise load (hours/week): 29.25 ± 9.48; lower-level group: 16.63 ± 6.82	The higher-level athletes had increased diversity and higher metabolic proportions of the gut microbiome for it may positively impact athletic performance	7 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies
Cronin, 2018, Ireland [55]	RCT	90	18–40	Male and female	Aerobic and resistance exercise training	Eight-week combined aerobic and resistance exercise training program	The improved body composition with exercise is not depending on basic changes in the diversity of microbial populations in the gut. The various microbial characteristics already observed in long-term habitual athletes may be a late response to exercise and fitness enhancement	5 (Cochrane risk-of-bias tool for randomized trials)
Kulecka, 2020, Poland [56]	Cross-sectional study	71	14–72	Male and female	Marathon running; cross-country skiing	Highest level of training endurance sports athletes trained on average 1.58 ± 0.58 times per day, taking 7.25 ± 2.17 training units in one week lasting 2.79 ± 1.74 h per day on an average	Excessive training is involved with the changes in composition and elevation of increased bacterial diversity	6 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies
Rettedal,2020, New Zealand [57]	Cross-sectional study	29	20–45	Male	HIIT training	Three weeks of high-intensity training (8–12 × 60 s cycle ergometer bouts at VO_2 maximum power output interspersed by 75 s rest, three times per week)	The gut microbiome's overall composition is not significantly altered by short-term HIIT, however some microbiome genera are linked to insulin sensitivity measures, and HIIT enhanced these markers in overweight subjects	5 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies

Table 2. *Cont.*

Author, Year, Country	Study Design	Sample Size	Age (Mean Value ± SD, Range/Age Group %)	Gender	Type of Exercise	Duration, Frequency, Training Volume	Outcome	Quality Assessment
Estaki, 2016, Canada [58]	Cross-sectional study	39	18–35	Male and female	Not specific	NA	Cardiorespiratory fitness is correlated with increased microbial diversity in healthy humans and that the associated changes are anchored around a set of functional cores rather than specific taxa	6 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies
Jang, 2019, Korea [59]	cross-sectional study	45	25 ± 3 (bodybuilders); 20 ± 1 (distance runners) Healthy men 26 (±2) years	Male	Bodybuilding; distance running	NA	Athlete type was significantly associated with the relative abundance of gut microbiota at the genus and species level: *Faecalibacterium*, *Sutterella*, *Clostridium*, *Haemophilus*, and *Eisenbergiella* were increased ($p < 0.05$) in bodybuilders, while *Bifidobacterium* and *Parasutterella* were decreased ($p < 0.05$). At the species level, intestinal beneficial bacteria widely used as probiotics (*Bifidobacterium adolescentis* group, *Bifidobacterium longum* group, *Lactobacillus sakei* group) and those were generating the short-chain fatty acids (*Blautia wexlerae*, *Eubacterium hallii*) were decreased in bodybuilders and the increased in controls	6 (Newcastle–Ottawa Quality Assessment Scale) adapted for cross-sectional studies
Barton, 2021, Ireland [60]	Cohort study	2	31.5	Male	Marathon and triathlon	Regular aerobic exercise complimented with twice weekly resistance training	Sustained fitness improvements support alterations to gut microbiota and physiologically relevant metabolites	6 (Newcastle–Ottawa Quality Assessment Scale—Cohort Studies)

The articles involved in the systematic review focusing on animal studies were published between 2017 [42] and 2021 [44,45] and show a very stable trend regarding the research in this field. One article was published in 2017 [42], one in 2019 [46], and two studies were published in 2021 [44,45]. The research in the animal field depicts a global dimension as the studies were conducted in various countries including one study in Finland [42], one study in the USA [43], one study in Taiwan [46], one study in China [44], and one study conducted in France [45]. In the systematic review papers, the specific study design was used to evaluate the results of all four studies conducted. On animals, the cohort study design was used to access the results [42,44–46]. The sample size used in the articles ranges from 20 subjects [42,45], to 30 subjects [44], to 100 subjects [46], and for animal studies, a wide range is required to access good results. The age number of the subjects ranges from 5 weeks [44] to 12 years [45], so it is a good factor to evaluate results in the small age group vs. mature groups. The subjects are 80% male and 20% female. Different physical exercises studied in the systematic review papers considering animals include exercises like exhaustive swimming in which weight is tied to the tail of mice to produce exhaustion during swimming [46]. In another study endurance and treadmill exercises were performed collectively to access the combined effect [42]. One study evaluated the results of endurance exercise on the gut microbiota [45] and the last study assessed treadmill exercises [44]. Thus, all these studies evaluated the results of these exercises in gut microbiota changes.

For the human studies, all the included articles were published between 2017 [43,47,48] and 2021 [49–52] and these studies were performed in several countries and show a very stable increasing trend. Three studies were published in China [48,53,54], two in the USA [43,48], one in Ireland [55], one in Poland [56], one in France [49], one in Portugal [50], one in Australia [51], one in New Zealand [57], one study in Canada [58], one in German [52], one in Korea [59], and one in Ireland [60]. The research related to the gut microbiota and its interaction with different types of physical activities has increased tremendously in recent years, showing a positive trend and interest in these terms depending on the health sector.

Different types of study designs used in the papers were included in the systematic review. The study design includes three randomized controlled designs [47,52,55], seven cross-sectional studies [43,48,54,56–59], four cohort studies [50,51,53,60], and one clinical trial [43]. In all the papers, the sample size ranges from 2 [60] to 90 [55], which is a very good ratio as the lowest and highest ranges are included in it. For the systematic review papers, the subjects included in these studies range from 12 years [53] to 72 years [56]. This impact of physical activity on gut microbiota covers a maximum age gap to deliver all the possible effects depending on the age groups. In total, 60% of the subjects in these articles are male, and 40% of the subjects are female.

For quality assessment, cross-sectional studies [43,48,54,56–59] were calculated by the New Castle–Ottawa Quality adapted for cross-sectional studies and the score was 83% fair with moderate risk of bias [48,54,57–59]. Cohort studies [42,44–46,49–51,53,60] were assessed by the Newcastle–Ottawa Scale for Cohort Studies (NOS) and the results showed low-risk bias in 45% of articles [42,49,51,53] and moderate risk in 55% [44–46,50,53,60]. Randomized clinical trials [47,52,55] were evaluated by the Cochrane collaboration risk-of-prejudice tool for randomized trials (RoB2) and the results showed a low-risk bias in two papers [52,55].

There are several types of physical activities and each type then alters the gut microbiota in its own way, so the articles included in the systematic review cover several types of athletes and sports including running [49,51,56,59], cycling [43] strength and endurance exercises [52], bodybuilding [59], rowing [53], skiing [56], football [50], aerobic and resistance training [55], martial arts [54], 51 km cross-country ski-march [47], and HIIT (high-intensity interval training) [57]. So, these studies presented a broad spectrum to evaluate diversity including all the possible physical activities.

Table 2 presents the papers encompassed in the meta-analysis, outlining the microbiota profiles and alterations resulting from physical training. It also classifies the levels of physical activity intensity for each group, control and experimental, across all studies.

In the pie chart, the designs of studies and the different levels of intensity investigated categorized by exercise intensity levels (low, moderate, vigorous) were summarized (Figure 2). These findings show the current research trends. In 30% of the studies included in the meta-analysis, authors performed the comparison between the same intensities [43,47,51,54], and in the 60%, the comparison was carried out between different levels of intensity [50,53,55,56,58,59], specifically in 40% low (less than 30% of VO_2 max) versus vigorous (more than 60% of VO_2 max), and 20% moderate (between 30% and 60% of VO_2 max) versus vigorous (more than 60% of VO_2 max).

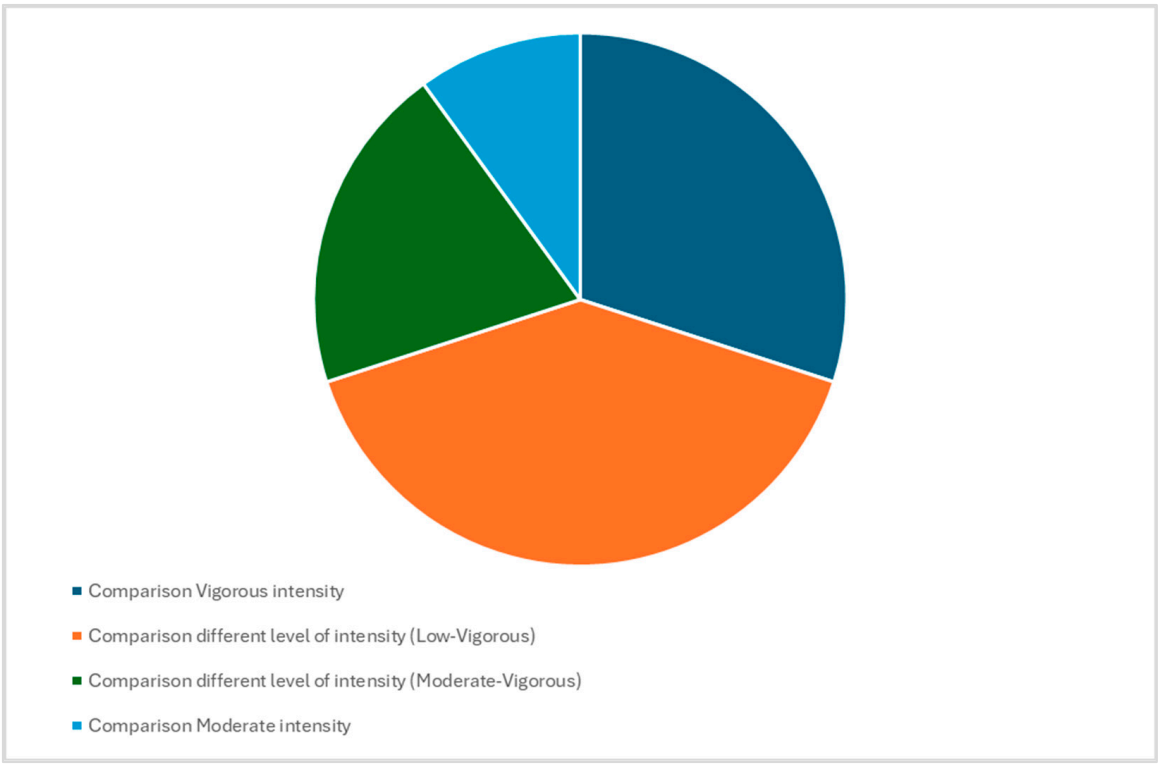


Figure 2. The pie chart illustrates the design of studies and the different levels of intensity investigated categorized by exercise intensity levels (low, moderate, vigorous). Specifically, for categorization, the following intensity levels were considered: low intensity (less than 3 METs or less than 30% of VO2 max); moderate intensity (between 3 and 6 METs or between 30% and 60% of VO2 max); vigorous intensity (more than 6 METs or more than 60% of VO2 max) [37–39].

The 10 studies selected for meta-analysis were performed on humans [43,47,50,51,53–56,58,59]. In 80% of the studies, fecal analysis is conducted after chronic adaptations from specific regular training over time [43,50,51,53–56,59], while the remaining 20% of analyses are performed post-training (immediately or until 1 week) [47,58]. All the studies were conducted on fecal samples using the Miseq platform Illumina technique, except one study [56] in which an ion torrent PGM sequencer was used. The V3–V4 regions of 16S amplicon sequencing are used in all the works, but different primers including the sequencing and universal primers are used. In all of the ten papers admitted in the meta-analysis, alpha and beta diversity was used to determine biodiversity, and in the eighth [43,47,50,51,53,54,56,58] of the ten papers, the Shannon index increased in the population performing high-intensity physical activities. They seem not modified in the presence of different aerobic physical activities or intensity.

The ratio, the B/B ratio index, was enhanced in subjects with physical activity intensities [53,56,59]. Specifically, in 50% of the studies (5 out of 10), there was an increase in the B/B ratio, and in four of these cases, it was associated with an increase in the intensity of training, ranging from low or moderate to vigorous, or with an increase in the volume of exercise load (hours per week) [43,47,53,56,59].

Further analysis using the stratification way of intensity of physical activity data (Figure 3) verified this trend. The meta-analysis was performed using the standardized mean difference as the results measure. A total of 10 studies were comprehended in the analysis, but two study are considered two different populations. The observed standardized mean differences ranged from −0.7583 to 3.5830, with most estimates being positive (83%). The estimated average standardized mean difference entrenched on the random-effects model was $\hat{\mu} = 0.7597$ (95% CI: 0.1764 to 1.3429). Therefore, the standard outcome varied significantly from zero (z = 2.5527, $p = 0.0107$). According to the Q-test, the true results appear to be heterogeneous (Q(11) = 170.5522, $p < 0.0001$, $\text{tau}^2 = 0.9827$, $I^2 = 94.4979\%$). A 95% prediction interval for the actual results is given by −1.2689 to 2.7882. Hence, although the average result is approximated to be positive, in some studies, the true results may in fact be negative.

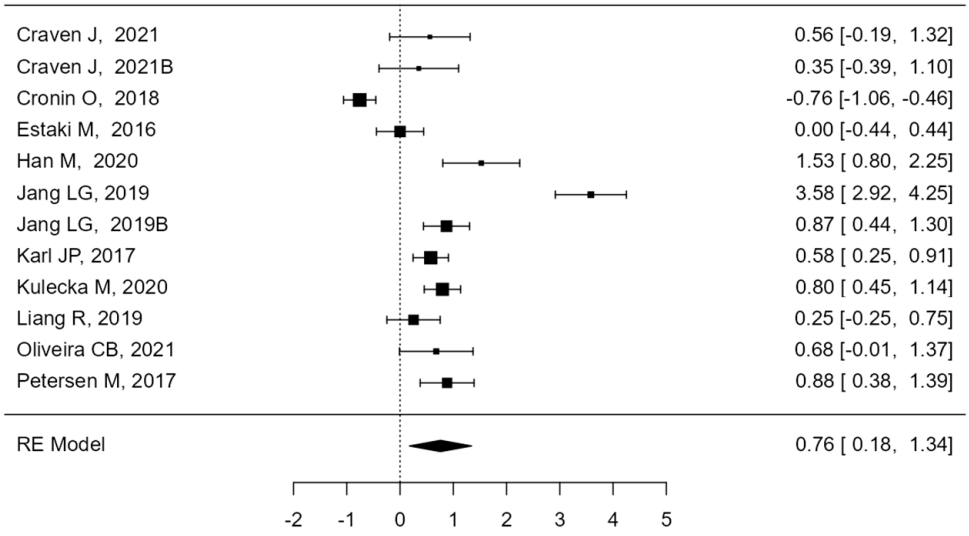


Figure 3. The results of the meta-analysis are shown in forest plot: the differences in the means of the Bacillota/Bacteroidota ratio for each study are analyzed [43,47,50,51,53–56,58,59].

The Bacillota/Bacteroidota ratio (formerly Firmicutes/Bacteroidetes), which describes gut health, was basically analyzed. In fact, it has been recommended as an achievable biomarker of dysbiosis since it has been described by several studies that there is a difference in this index among normal weight and obese individuals. Consequently, the B/B ratio is often cited in the scientific literature as an indication of obesity or less-than-good health states, although the scientific community is not unaware of this definition. In the present study, as shown in Table 3 and Figure 3, the B/B ratio changed based on how various levels of intensity of exercise impacted the gut microbiota ratios.

Table 3. Characteristics of samples and specificities of included studies in meta-analysis.

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Craven, 2021, Australia [51]	↔Alpha-diversity (Shannon index, Chao1)	Microbial communities are similar	(A) CON = 1.3; PA = 0.7; (B) CON = 1.2; PA = 0.8	High-volume training: ↓ *Lachnoclostridium* ($p = 0.02$), *S. parasagunis* ($p = 0.02$) ↑ *R. callidus* ($p = 0.03$) *Lachnoclostridium* (bacillota) 21% *H. parainfluenzae* (pseudomonadota) 0.5% *S. parasanguinis* (bacillota) 3% *R. callidus* (bacillota) 15%	NA	Only three types of Bacillota identified including *Lachnoclostridium*, *S. parasanguinis* and *R. callidus*. No bacteroidota present. Overall in Bacillota only *R.callidus* percentage increased after high volume and taper training as compared to normal training while *Lachnoclostridium* and *S. parasanguinis* percentage decreased in high volume and taper training	Faecal samples were obtained before and instantly after each training (three weeks of the normal training, three weeks of the high-volume training and a one-week taper training)	*Pasteurellaceae* (pseudomonadota) 0.5% *Haemophilus* (pseudomonadota) 0.5% *Lachnoclostridium* (bacillota) 21% *H. parainfluenzae* (pseudomonadota) 0.5% *S. parasanguinis* (bacillota) 3% *R. callidus* (bacillota) 15%	Middle distance running CON = Normal training; PA = Higher level training	(A) Vigorous (60–84% VO_2R) (B) Vigorous (60–84% VO_2R) + Normal Training volume	(A) Vigorous (60–84% VO_2R) (B) Vigorous (60–84% VO_2R) + Training volume increasing during the activity	(A) Vigorous > 6 METs; (B) Vigorous > 6 METs	(A) Vigorous > 6 METs; (B) Vigorous > 6 METs
Cronin, 2018, Ireland [55]	α-diversity Shannon index changes were non-significant (no α-diversity value provided)	Microbial communities are statistically different. Bray-Curtis's dissimilarity β-diversity changes were non-significant (no β-diversity value provided)	CON = 0.02; PA = 0.0015	*Borrelia hermsii* (Spirochaetota) 2.3% *Mycoplasma pneumoniae* (Tenericutes) 6.6%; *Streptococcus thermophilus* 0.002%	NA	In training group the Bacillota are *Streptococcus thermophillus* and *Lactobacillus lactis*. and in one training group Bacillota are only *Streptococcus thermophillus*	Fecal samples collected before and after 8 weeks intervention (exercise only group, exercise and protein supplement group and control group)	Only exercise group: *Borrelia hermsii* (Spirochaetota) 2.3%; *Mycoplasma pneumoniae* (Tenericutes) 6.6%; only protein group: *Streptococcus thermophilus* 0.002%	Aerobic and resistance exercise training CON = Sedentary; PA = exercise	Low (20–39% VO_2R)	High (60–84% VO_2R)	Vigorous > 6 METs (METs consumption related to daily life)	Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Estaki, 2016, Canada [58]	↑ * Shannon, Chao1, Simpson ↑ Alpha diversity (Chao, Shannon, Simpson)	Microbial communities are statistically different. Beta diversity (Bray–Curtis)	CON = 1.4; PA = 0.53	↑ * *Lachnospiraceae* ↑ * *Christensenellaceae*, ↑ * *Ruminococcaceae* ↑ * *Clostridiales*. (p = 0.026), *Roseburia*, *Lachnospiraceae*, *Erysipelotrichaceae*	NA		Fecal samples were collected in healthy adults before and after one week of VO_2 testing	*Erysipelotrichaceae*, *Coprococcus*, *Roseburia*, *Adlercreutzia*, and unknown members of *Clostridiales*, *Lachnospiraceae*, and *Erysipelotrichaceae*.	CON = lower level exercise; PA = higher level exercise	Low (20–39% VO_2R)	High (60–84% VO_2R)	Vigorous > 6 METs (METs consumption related to daily life)	Vigorous > 6 METs
Han, 2020, China [53]	↑ * Shannon, Simpson ↑ Alpha diversity (Shannon, Simpson)	Microbial communities are statistically different. Beta diversity (Jac card, Unifrac), ↑ B/B	CON = 2.31; PA = 3.89	↑ * *Ruminococcaceae*, *Clostridiales*, *Faecalibacterium* and *Lachnospiraceae*.	↑ *	Bacillota included are *Ruminococcaceae*, *Clostridiales*, *Faecalibacterium* and *Lachnospiraceae*.	Fecal samples were collected from adult elite athlete's (AE), youth elite athlete's (YE), and youth non-elite athlete's (YN) during two months period	The average relative abundances of Bacillota and *Proteobacteria* of AE (76.27% and 8.73%) and YE (64.7% and 10.69%) were higher than those of YN (58.12% and 8.01%), the moderate relative richness of *Bacteroidota* in YN (26.19%) was significantly increased than that of AE (11.41%) and YE (16.63%). *Ruminococcaceae_unclassified*, *Clostridiales_unclassified*, *Faecalibacterium* and *Lachnospiraceae_unclassified* were highest in the AE cohort, and *Bacteroides* and *Prevotella* were prominent in the YE cohort and YN cohort	Rowing CON = non elite athletes; PA = elite athletes	Moderate (40–59% VO_2R)	Vigorous (60–84% VO_2R)	Vigorous > 6 METs	Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Jang, 2019, Korea [59]	↓ * Shannon, OTUs, Chao1 Diversity between groups: → Alpha diversity (Chao1) ≠ Beta diversity (PC) Alpha and beta diversity remains same in both groups	Microbial communities are statistically different	(A) CON = 4.79; PA = 15.69; (B) CON = 4.79; PA = 3.96	*Faecalibacterium*, *Sutterella*, *Clostridium*, *Haemophilus*, and *Eisenbergiella* were increased in ($p < 0.05$) in bodybuilders, while *Bifidobacterium* and *Parasutterella* were the decreased ($p < 0.05$)	NA	The Bacillota compromised of *Blautia wexlerae*, *callidus*, *Faecalibacterium_uc Faecalibacterium prausnitzii*, *Clostridium innocuum Eubacterium hallii*, *Ruminococcus*, *Weissella confusa*, *Lactobacillus sakei*. The Bacteroidota compromised of *Bacteroides stercoris* and *Bacteroides caccae*	Fecal samples were obtained from sedentary men as control and from bodybuilders and distance runners	*Faecalibacterium*, *Sutterella*, *Clostridium*, *Haemophilus*, and *Eisenbergiella* were the highest ($p < 0.05$) in bodybuilders, while *Bifidobacterium* and *Parasutterella* were the lowest ($p < 0.05$). At the species level, intestinal beneficial bacteria widely used as probiotics (*Bifidobacterium adolescentis* group, *Bifidobacterium longum group*, *Lactobacillus sakei* group) and those producing short-chain fatty acids (*Blautia wexlerae*, *Eubacterium hallii*) were the lowest in bodybuilders and the highest in controls	Body building; distance running (A) CON = not exercise; PA = body-building; (B) CON = not exercise; PA = distance running	(A) Low (20–39% VO_2R) (B) Vigorous (60–84% VO_2R)	(A) Vigorous (60–84% VO_2R) (B) Vigorous (60–84% VO_2R)	(A) light < 3 METs (B) Vigorous > 6 METs	(A) Vigorous > 6 METs (B) Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Karl, 2017, USA [47]	↑ * Shannon, Chao1 ↑ Alpha diversity (Shannon) → Alpha-diversity (Chao1, OTU) ↑ B/B ratio	Bray-Curtis distances	CON = 1.28; PA = 1.86	*Pepto-streptococcus*, *Chris-tensenella*, *Faecalibac-terium*, *Staphylococ-cus*, unassigned taxa within the *Mogiobac-teriaceae*, *Christensenel-laceae*, *and Planococcaceae families*. *Verru-comicrobia* *, *TM7* *, *Tenericutes* *, *Spirochaetes* *, *Proteobacteria*, *Lentisphaerae* *, *Fusobacteria* *, *Bacillota* *, *Eu-ryarchaeota* *, *Cyanobacteria*, *Bacteroidota* *, *Actinobacteria*	↓ *	↑ *	Fecal samples were collected from subjects the control group, the protein sup-plemented group and carbohydrate supple-mented group two days before and one day after the stress exercises	*Pepto-streptococcus*, *Christensenella*, *Faecalibacterium*, *Staphylococcus*, unassigned taxa within the *Mogiobacteriaceae*, *Christensenellaceae*, and *Planococcaceae* families. *Verrucomicrobia* *, *TM7* *, *Tenericutes* *, *Spirochaetes* *, *Proteobacteria*, *Lentisphaerae* *, *Fusobacteria* *, *Bacillota* *, *Euryarchaeota* *, *Cyanobacteria*, *Bacteroidota* *, *Actinobacteria*	Fifty-one kilometer of cross-country ski-march CON = pre-training; PA = post-training	Vigorous (60–84% VO_2R) [61]	Vigorous (60–84% VO_2R) [61]	Vigorous > 6 METs	Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Kulecka, 2020, Poland [56]	Chao1, Simpson (p = 0.025 & p = 0.00059) ↑ Alpha diversity (Shannon, Simpson, Chao1), ↑ B/B ratio	Microbial communities are statistically similar	CON = 1.83; PA = 10.04	*Bacteroides*, *Prevotella*, *Alistipes*, *Sutterella* and *Subdoligranulum*, *Ruminococcaceae* family and *Barnesiella* genus, *Bacillota*; *Clostridia*; Clostridiales; *Lachnospiraceae*; *Lachnoclostridium*; *Proteobacteria*; *Gammaproteobacteria*; *Pasteurellales*; *Pasteurellaceae*; *Haemophilus*; *Bacteroidia*; *Bacteroidales*; *Prevotellaceae*; *Prevotella_9*	*Bacteroidota* ↑* *Prevotella*		Stools samples were obtained from the 14 marathon runners, 11 cross country skiers and the 46 inactive healthy controls	*Bacteroides*, *Prevotella*, *Alistipes*, *Sutterella* and *Subdoligranulum*, *Ruminococcaceae* family and *Barnesiella* genus, *Bacillota*; *Clostridia*; *Clostridiales*; *Lachnospiraceae*; *Lachnoclostridium*; *Proteobacteria*; *Gammaproteobacteria*; *Pasteurellales*; *Pasteurellaceae*; *Haemophilus*; *Bacteroidia*; *Bacteroidales*; *Prevotellaceae*; *Prevotella_9*	Marathon running; cross-country skiing CON = lower level exercise; PA = higher level exercise	Low (20–39% VO_2R)	Vigorous (60–84% VO_2R)	(A) light < 3 METs	Vigorous > 6 METs
Liang, 2019, China [54]	↑* Shannon (p = 0.019), Simpson (p = 0.001)	Microbial communities are statistically different	CON = 1.9; PA = 0.568	↑* *Parabacteroides* (p < 0.001) *Oscillibacter* (p = 0.026 *Bilophila* (p = 0.036) *Megasphaera* (p = 0.04) *Phascolarctobacterium* (p = 0.028)	↑* Megasphaera (p = 0.04)	*Veillonellaceae*, *Phascolarctobacterium*, *Oscillibacter* and *Megasphaera*. The *Bacteroidota* are *Porphyromonadaceae* and *Parabacteroides*	Fecal samples were collected from 12 large scale and 16 small scale athletes	Higher level group: *Porphyromonadaceae* 4.4%; *Veillonellaceae* 0.9%; *Parabacteroides* 2.3%; *Phascolarctobacterium* 2.2%; *Oscillibacter* 0.7%; *Megasphaera* 0.008%; *Bilophila* 0.3%; Lower-level group: *Porphyromonadaceae* 2%; *Veillonellaceae* 4.5%; *Parabacteroides* 0.8%; *Phascolarctobacterium* 0.5%; *Oscillibacter* 0.3%; *Megasphaera* 0.069%; *Bilophila* 0.075%	Professional martial arts CON = Lower level group; PA = Higher level group	Moderate (40–59% VO_2R) + Lower-level of Exercise load (hours/week)	Moderate (40–59% VO_2R) + Higher level of Exercise load (hours/week)	Vigorous > 6 METs	Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Oliveira, 2021, Portugal [50]	↑ * Shannon, Chao1, Simpson (p = 0.013)	Microbial communities are statistically different	CON = 1.78; PA = 1.677	↑ * *Collinsella aerofaciens* ↑ * *Faecalibacterium prausnitzii*	↑ * *Prevotella Copri* (31%)	*Bacillota* included are *Fecalibacterium* and *Bacteroidota* included is *Prevotella*	Fecal samples were collected from subjects on 2nd, 3rd, 9th and 10th day of matches	At the beginning, 50% of bacteria were Bacillota (Frimicutes) followed by Bacteroidota (28%) and Actinobacteria (19%). Bacillota (Frimicutes) (52%) was the most prevalent type overall at the conclusion of the event followed by Bacteroidota (31%) and Actinobacteria (14%). At baseline, Faecalibacterium (29%) was the most prevalent bacterial genus followed by Collinsella (16%) and Prevotella (13%). At the conclusion of the competition Faecalibacterium was the most prevalent overall (29%) followed by Prevotella (17%) and Collinsella (12%). Faecalibacterium prausnitzii was the most prevalent by the end of the competition (30%) followed by Collinsella aerofaciens (13%) and Prevotella copri (12%)	Football CON = Baseline; PA = End of study	Moderate (40–59% VO_2R)	Vigorous (60–84% VO_2R)	Vigorous > 6 METs	Vigorous > 6 METs

Table 3. *Cont.*

Author, Country, Year [Ref]	Biodiversity Indicators, Alpha Diversity	Biodiversity Indicators, Beta Diversity	B/B Ratio	Bacillota	Bacteroidota	Diversity	Comparison between Groups	Bacillota/ Bacteroidota	Type of Exercise and Control	Classification of Exercise and Sport Intensity Based VO_2 Max [38] and Details		Classification of Exercise and Sport Intensity Based METs Values [39] and Details	
								%		CON	PA	CON	PA
Petersen, 2017, USA [43]	↑ * Shannon (p = 0.0004), OTUs	Microbial communities are statistically different	CON = 2.05; PA = 2.33	NA	↑ * *Prevotella*		Fecal samples were obtained from 33 cyclists with extensive medical issues and no antibiotic use within the last year	NA	Cycling PA = professional level (the highest level) and CON = amateur level	Vigorous (60–84% VO_2R) + lower level of Exercise load (h/week)	Vigorous (60–84% VO_2R) + higher level of Exercise load (h/week)	Vigorous > 6 METs	Vigorous > 6 METs

↑ = increase,↑* = statistically significant increase, ↓ = decrease, ↓* = statistically significant decrease, ↔ = no differences between groups, CON = control, PA = physical exercice, NA = not available.

An examination of the residuals revealed that one study [59] had a value larger than ±2.8653 and maybe a potential outlier in the context of this model, which could be overly influential [59]. There was no evidence of funnel plot asymmetry in either the regression test or the rank correlation (p = 0.1256 and 0.6384, respectively; Figure S1). Finally, to find the effect of moderator variables on the B/B ratio, subgroup analyses and me-ta-regressions were performed. We performed a meta-regression of the incorporated intervention types (type—aerobic or anaerobic, intensity or duration), sample size, the publication year, the methodological quality of the study, and the WHO region origin. The meta-regression only revealed that the type of treatment had an impact on the Bacillota/Bacteroidota ratio in adults (p = 0.004) and the intensity of exercise determines these ratios. The meta-regression revealed that the type of treatment had an impact on the B/B ratio in adults (p = 0.004) and the intensity of exercise determines these ratios. This was also seen, but only if associated with vigorous intensity, for the duration (p = 0.410).

4. Discussion

The importance of the gut microbiota on human health and sports performance has come to light in recent decades. Different physical activity patterns can have different effects on the gut microbiota and, in turn, on athletic performance. Here, in this systematic review and meta-analysis we examine how various forms and intensity levels of physical activity and their impact on microbial diversity.

Specifically, in the qualitative systematic review emerge different important aspects from the included studies. In their 2021 study, Moitinho–Silva et al. [52] explored the interaction of different physical activities like strength exercises and endurance exercises and found that typologies of exercise have different but moderate impacts on the overall human physiology and very distinct microbial modifications in the gut. They involve subjects practicing endurance and strength training compared to controls who did not exercise regularly. As a result, the physically active group shows significantly more of the genera *Coprococcus*, *Parasutterella,* and the family *Ruminococcaecae* in comparison to the control group. In 2019, Jang et al. [59] performed a cross-sectional study using bodybuilders and distance runners, providing a high-protein, high-fat, low-carbohydrate, and low-fiber diet to bodybuilders and a low-carbohydrate and low-fiber diet to distance runners. The athletes consuming a high-protein and low-carbohydrate diet had decreased diversity of gut microbiota and decreased abundance of short-chain fatty acid-producing bacteria, which depicts the role of diet alongside physical activity to affect gut microbial diversity. In 2017, Karl et al. investigated military training effects using a randomized controlled trial in three different groups, provided with protein-based and carbohydrate-based diets and 4 days arctic military training [47]. They observed that physiological stress influences intestinal permeability and modifying diet changes gut microbiota composition. Moreover, the Shannon and chao1 indices showed an increasing trend [47].

In 2020 cohort research conducted by Han et al. [53], the gut microbial communities of elite and non-elite athletes at the juvenile and adult levels were observed to see how they vary on the type of exercise in relation to nutritional variables and physical attributes. Kulecka et al. [56] conducted a cross-sectional study on cross-country skiers and marathon runners who had undergone the most intense endurance training. They found that intensive training is linked to both compositional changes and the growth of greater bacterial diversity. Additionally, athletes' enhanced taxa are known to be involved in the fermentation of fiber. In 2017, Yang et al. conducted a cross-sectional study [48] wherein premenopausal women were assessed for VO_2-max. The findings indicated that gut microbiota composition and cardiorespiratory fitness are related, independent of age and carbohydrate or fat intake. The relationship between VO_2-max and gut microbiota, on the other hand, seems to be mediated by body fatness and physical activity.

In 2021, Tabone et al. [49] performed a clinical trial on cross-country runners and involved them in performing different treadmill exercises at different intensities, the results depict that the performance of an individual exercise bout, in cross-country non-

professional athletes, shows significant changes in the microbiota, in serum and fecal metabolome, which may have the health implications. In 2021 Oliveira et al. [60] conducted a cohort study in which the perceived exertion method was applied on female football players to observe the changes in gut microbial diversity before and after the football tournament. The gut microbiota composition of elite female footballers was not altered by the physical and physiological demands of training and matches in an official international tournament. Although the Shannon, chao1, and Simpson indices increased and the Bacillota and Bacteroidota did not change much before and after the tournament. In 2018, Cronin et al. [55] observed in athletes performing aerobic and resistance exercise training for 8 weeks that the enhanced body composition with exercise does not rely on major changes in the composition of microbial populations in the gut.

In 2021, Barton et al. [60] performed a 6-month cohort study on male marathon and triathlon runners, involving them in regular aerobic exercise complimented with twice-weekly resistance training, and observed alterations in gut microbiota and physiologically relevant metabolites due to sustained fitness exercises. In 2021, Craven et al. [61] conducted a 7-week cohort study on middle-distance runners and different training volumes to observe the association with gut microbiota. The results revealed that the alpha-diversity and global composition of the gut microbiome were unchanged by modifications in training volume.

In 2021 [57], Rettedel et al. performed a cross-sectional study in which 3 weeks of HIIT training were used to observe the influences on intestinal microbiota. Short-term HIIT does not impact the gut microbiome, but some genera are linked with some metabolic process in participants.

In 2016, Estaki et al. conducted a cross-sectional study and observed a correlation between improved cardiovascular fitness and greater microbial diversity in healthy humans, with the associated alterations anchoring around a set of functional nuclei rather than a particular taxonomic group [58]. In 2019, Liang et al. [54] performed a cross-sectional study on two groups of martial art athletes, group 1 performing high-level exercise and group 2 with low-level exercise. They were observed for any changes in gut microbiota depending upon the intensity of physical activity. The results indicate the increased diversity and higher metabolic capacity of the gut microbiome in group 1 as compared to group 2. According to Karl et al. [47], a good increasing trend on the ratio of Bacillota to Bacteroidota and increasing trend in proteobacteria and actinobacteria were observed. Also, stress was associated with intestinal permeability and changes in gut microbiota by modifying diet. In a study conducted by Han in 2020, the gut microbial communities depend upon the type of exercise associated with dietary factors and physical characteristics [53]. In 2021, Mach et al. [45] performed a study on horses, in which he modulated the level of physical activities and targeted the gut-mitochondria axis, and it appears to be a potential strategy to enhance horses' physical performance.

Here, a meta-analysis was conducted using ten studies as the focus, based on the findings from the narrative reviews that were already available or from other systematic review reviews. As previously reported in the literature, increasing exercise training was observed to affect the composition of the gut microbiota, particularly decreasing Bacteroidota (formerly Bacteroidetes), and increasing Bacillota (formerly Firmicutes), indicating a shift in the B/B ratio [62–64]. The B/B ratio tended to enhance in subjects with high-intensity levels (vigorous, 60–84% VO_2R), as suggested in previous works [62–64]. The Bacillota/Bacteroidota (B/B) ratio, which is often used to describe gut health, is one of the indicators considered in this study. In fact, in some studies, results have described the B/B ratio and shown that it undergoes variations in athletes versus sedentary individuals, proposing it as a possible biomarker of alteration of the health status of the gut [22]. According to the previous study, it was illustrated that the B/B ratio tends to decrease in subjects with sedentary or low physical activity [5]. Bacteroidota significantly decreased in response to the intensity of exercise, according to our findings. This change implies that this phylum, which is an essential component of the gut microbiota, may decline

because of exercise therapies. It is well known that bacteria play a role in the complicated digestion of carbohydrates and the consumption of energy. The decline that is seen following exercise initiates the question of what influences PA specifically on this group and its implied metabolic counter; hence, diet modification is necessary. Contrary to expectations, Bacillota, a prominent phylum of gut microbiota, had a noteworthy rise following physical activity. This rise implies that exercise therapies may have a positive effect on Bacillota in the gastrointestinal tract. The gut metabolic processes may be linked to an increase in the number of bacteria that play a role in energy birth and storage. The ability to engage in endurance exercise indirectly strengthens the intestinal barrier. The gut barrier is indirectly strengthened by endurance exercise capacity. *Lactobacillus* (Bacillota) and *Bifidobacterium* have been demonstrated repeatedly to improve intestinal barrier integrity through a variety of methods, including greater capacity for butyrate synthesis. As a result, there is less migration of endotoxins and germs from the intestinal lumen into the systemic circulation, which lowers inflammation and strengthens immunity. The release of pro-inflammatory mediators, including lipopolysaccharide (LPS) and tumor necrosis factor-alpha (TNF-α), has demonstrated that healthy gut microbiota release a small quantity of LPS into the bloodstream, which is vital for the maintenance and development of the host immune system, and different pathophysiological reactions within various organs, including adipose tissue, the liver, and the endothelium [65,66]. Furthermore, the anti-inflammatory effects, which are typically accompanied by the release of cytokines and peptides, also exert an influence on skeletal muscles [67]. Thus, it is plausible that the increase of *Lactobacillus* enhances the integrity of the intestinal barrier and improves aspects of host health that have been associated indirectly with increasing the capacity for endurance exercise. The significant rise that is seen in response to exercise emphasizes the beneficial role that PA plays in regulating this phylum, possibly promoting greater overall health and metabolic balance. Furthermore, the Bacillota phylum encompasses genera that are capable of producing short-chain fatty acids (SCFAs) [67,68]. Short-chain fatty acids (SCFAs) are widely recognized for their involvement in crucial processes such as maintaining colonocyte integrity, enhancing barrier function, and increasing mucin expression through the regulation of specific gene expression [68,69]. Moreover, the scientific evidence supports the notion of an inverse relationship between the type and intensity of physical activity and the quantity of fecal bile acids that influence the increase in the Bacillota (mainly *Clostridia* spp.) [70]. Collectively, these observations reinforce the concept that gut bacteria play an active role in metabolic homeostasis (Figure 4) [11,71].

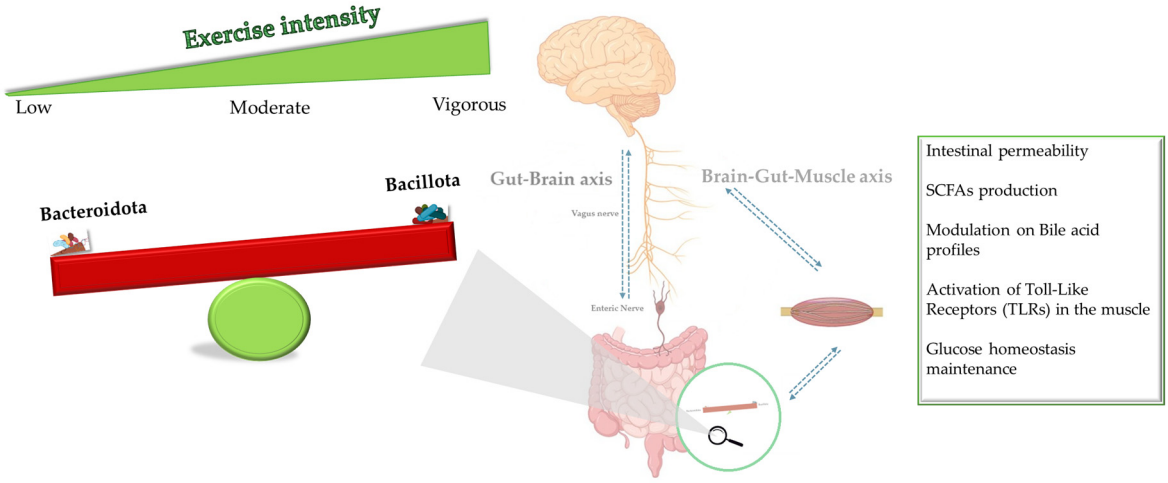


Figure 4. Variation in the Bacillota/ Bacteroidota ratio can occur based on physical intensity training and allow, in this way, our body to respond to intense exercise with several physiological and metabolic mechanisms.

This systematic review and meta-analysis have certain limitations. First off, the sociodemographic traits and dietary practices of the participants in the selected studies varied significantly, which restricts their comparability and may have an impact on the consistency of the findings. In addition, the studies analyzed varied in duration and frequency, which limited their comparability and the characterization of the effects of time and exercise volume. There were variations in the study's quality as well, but the main problem with the quality and the robustness of the conclusions was the absence of controls over confounding variables. However, this systematic review and meta-analysis offers new perspectives for future research into the changes in bacterial composition and is a first step in meticulously characterizing the different forms of biodiversity observed in athletes' gut microbiota.

5. Conclusions

The available data indicate that the microbiota can be affected by the level of physical intensity, with implications for bacterial abundance and diversity indices. These findings have implications for the physiological and metabolic system. Our meta-analysis indicates that the Bacillota/Bacteroidota ratio tends to increase in relation to levels of physical activity intensity. This suggests that Bacillota may play a specific role in adaptation to higher levels of physical activity. In future, specific study of the role of individual species could lead to intervention strategies aimed at stimulating beneficial pathways for athletes. It is therefore imperative to increase our knowledge of the interaction between the gut microbiota and physical activity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/sports12080221/s1, Table S1. PRISMA Checklist. Figure S1. The funnel plot shows the distribution of studies for publication bias.

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The sex related differences in health and Disease: A systematic review of sex-specific gut microbiota and Possible implications for microbial pathogenesis

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ABSTRACT

Background: The gut microbiota, a complex ecosystem influenced by various physiological and environmental factors, has been increasingly recognized for its role in health and disease. Emerging evidence suggests that sex differences, particularly mediated by sex hormones and physiological variations, significantly influence the composition and diversity of the gut microbiome. This systematic review aimed to evaluate and synthesize the current knowledge on sex-related variations in gut microbiota across human and animal studies.

Methods: We conducted a systematic review of 24 eligible studies, selected from an initial 13,205 articles, focusing on healthy populations and next-generation sequencing-based microbiota profiling in both humans and animal models.

Results: The results reveal sex-specific differences in microbial diversity and taxa abundance; however, the consistency and significance of these findings vary across studies, with females generally exhibiting higher levels of *Akkermansia* and *Bifidobacterium*, while males showed increased levels of *Prevotella* and *Escherichia*. These findings suggest that sex may be a contributing, but not necessarily dominant, biological variable shaping microbiome architecture across various species, including mice, pigs, deer, and humans, and highlight the influence of hormonal fluctuations, body composition, and lifestyle factors on gut microbial ecosystems.

Conclusion: Our findings underscore the importance of considering sex as a key biological variable in microbiome research and its potential implications for disease susceptibility, therapeutic interventions, and microbiome-targeted strategies in microbial pathogenesis. Moreover, evidence from human studies remains limited, especially those using 16S rRNA gene sequencing, which may lack the resolution to detect strain-level or functional differences. Incorporating multi-omics approaches such as metagenomics, metatranscriptomics, and metabolomics may offer deeper insights into sex-dependent microbial dynamics.However, these implications remain largely associative and require mechanistic validation in future studies.

1. Introduction

A variety of microorganisms inhabit different areas of the human body, including the skin, mouth, and vagina, with the majority found in the gut. The term "gut microbiota" describes all microorganisms located in the gastrointestinal tract (GI), primarily consisting of bacteria along with some viruses and fungi [1]. It is estimated that around 1000 to 1150 species of bacteria can thrive in the human colon, with each person hosting a minimum of 160 species [2]. The gut microbiota also function as a distinct endocrine organ; by producing, modulating, and metabolizing a variety of hormones and hormone-like compounds, such as serotonin, dopamine, and short-chain fatty acids, that contributes to the preservation of energy balance and the activation of the host's immune system through ongoing molecular interactions with the host [3]. Research involving humans and germ-free mice has demonstrated the gut microbiota's functional significance in enhancing health outcomes [4,5].

Since the gut microbiota plays a crucial role in maintaining the host's homeostasis and contributes to immune and metabolic functions, any disruption in this microbiota (dysbiosis) is linked to not only gastrointestinal diseases but also other conditions, including cancers, metabolic disorders, allergies, and immune system issues. Recent advancements in

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sequencing technology have led to a significant increase in data concerning gut microbiota [6,7]. Various factors, such as diet, ethnicity, age, antibiotic use, stress, psychological conditions, maternal health, delivery methods, environmental influences, and physical activity, can affect the composition of the gut microbiota [8,9]. Since the gut microbiome plays a role in both excretion and circulation, the concept of the interplay between sex hormones and the gut microbiome, is a developing area of research [10,11].

Along with these factors, studies carried out on human beings and animals discussed the importance of sex as a major contributor to the gut microbiota variability [12]. The role of sex in the percentage of different strains of gut microbiota in the human colon remains a topic of discussion. In 2011, Aguirre et al., [13] conducted a study on 10 healthy individuals from Australia (5 males and 5 females), discovered that men had a higher level of colonic mucosa-associated *F. prausnitzii* compared to women. A study conducted by Davis et al., has shown that the composition of microbiota differs between male and female mice [14]. One investigation found that transferring fecal microbiota from male mice to female mice resulted in elevated testosterone levels in the females. Collectively, these findings illustrate the influence of estrogen and testosterone on microbial ecosystems. In fact, it seems that these hormones may be responsible for these alterations, as germ-free (GF) mice exposed to female human fecal suspensions exhibited microbial species clustering in relation to the sex of the animals [15]. Moreover, overall body fat content appears to affect microbiota composition [16,17], thereby accentuating sex-related differences.

Nevertheless, there is still a limited understanding of how sex influences the gut microbiota in both humans and experimental animals, and even less is known about the mechanisms behind this influence. Consequently, these elements should be considered when designing a microbiota study and when analyzing the findings; however, this aspect has often been overlooked by researchers despite its relevance. To support this, we compiled the existing knowledge on the sex-dependent differences in gut microbiota of humans and different animals over their lifespans in this review. We examine how developmental phases and external factors, such as diet, age, and geographic location, relate to sex and specifically aim to assess the effects of sexual hormones.

1.1. Aim

The objectives of this review wereto compare the microbiota-associated differences in males and females in both human and animal studies and to assess potential factors and sex hormones that might affect microbial diversity and could be the underlying cause of the observed variations related to sex-differences.This systematic review was conducted to assess the existing research on the relationship between sex hormones and microbial composition and diversity in human and animal subjects.

2. Methods

This systematic review was conducted following the guidelines by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.

2.1. Selection protocol and search strategy

The review was registered with the PROSPERO International Prospective Register of Systematic Reviews (CRD420251020857) (http://www.crd.yorkac.uk/PROPSERO). The Review methods were developed using the Cochrane Handbook for Systematic Reviews of Interventions and the Preferred Reporting Items for Systematic Reviews and meta-analysis guidelines were used for reporting the methods and outcomes. This review involved a search of the Scopus, Google Scholar and PubMed data sources for all articles published in the English language between 2006 and 2024, investigating the effect of sex differences on gut microbial diversity in humans and animals. Particularly, the review question was framed using the PICOS framework and the following eligibility criteria were used: (a) Population: Healthy subjects; (b) Intervention: No intervention used; (c) Comparison: age- and sex-matched control group; (d) Outcome: sex differences in gut microbiota; (e) evaluation of differences in α-diversity and β-diversity, relative abundance of particular bacteria, and/or metagenomic information analyzed through 16S amplicon sequencing; (f) papers written in English; (g) observational studies (cross-sectional, cohort, case-control and nested case-control studies and experimental study design). Clinical trials, reviews, meta-analyses, case studies, case reports, proceedings, qualitative studies, commentary studies, and studies lacking a control group, in addition to those involving unhealthy participants and featuring incomplete designs, were not included. Studies investigating the association between estrogen and/or testosterone on the composition and/or diversity (alpha and/or beta) of the human gut microbiota in the male and/or female subjects were eligible for inclusion. Only studies that utilized next-generation sequencing (NGS), including shotgun sequencing or 16S rRNA amplicon sequencing, and were independent of culture-based techniques, to analyze the stool samples were included. Moreover, research studies focusing on the microbiota of the skin, oral cavity, stomach, or nasopharynx were not included.

The databases PubMed, ScienceDirect, and Scopus were utilized with the search terms labeled as Study: All studies. The search terms employed (whether combined or individual) were as follows: PubMed, ScienceDirect, and Scopus databases were accessed using the following terms (“gut microbiota, sex, gender” [MeSH Terms] OR (“microbiota” [All Fields] AND “gender” [All Fields] AND “sex” [All Fields]) OR “human gut microbiota” [All Fields] OR (“microbiota” [All Fields] AND “Gut” [All Fields] AND “sex” [All Fields] AND “gender” [All Fields])) AND (“microbiota” [MeSH Terms] OR “microbiota” [All Fields] OR “microbiotas” [All Fields] OR “microbiotas” [All Fields] OR “microbiota” [All Fields] OR “microbiome”). The search string used on PubMed was carried out using title, abstract, and MeSH terms; the search on Scopus and Web of Science involved content by title, abstract, and keywords. The screening of studies consisted of a multi-step rejection process, with four experts personally reviewing the titles and abstracts of the studies [18].

2.2. Data analysis

After reviewing the title and abstract of the eligible studies, we classified the articles into the following two categories depending upon the characteristics. Accordingly, we present the results of eligible studies based on each category.

2.3. Inclusion and exclusion criteria

Inclusion criteria for this review were 1) studies published in last 20 years which are designed to evaluate the differences in the gut microbial diversity based on sex differences, 2) studies that investigated the microbial community’s prevalence in male and female populations of humans and animals, 3) studies that reported impact of sex differences on different gut microbiota. All the subjects included in the studies are healthy and no specific age criteria was outlined, in order to select a wide range of data. Outcome measures included changes in at least one of the following: Anthropometric measurements, intestinal microbiota markers, dietary consumption data, and changes in sex hormones. Studies were excluded if the impact is not identified based on sex variation and if the population is unhealthy subjects, as sex difference was an important factor.

2.4. Data synthesis

All results gathered were transferred to the EndNote library for the selection phase, during which the primary author evaluated the articles

by reading the titles and abstracts to identify and eliminate duplicates. Moreover, the primary author assessed all the remaining titles and abstracts against the inclusion criteria. Articles deemed ineligible were discarded, and the reasons for their exclusion (not a study; incorrect study population; not a sex-based approach; or not relevant outcome measures) were documented. For differences in the overall content of the gut microbiota, we only performed a descriptive literature review because of the different situations of determination for the proportion of microbiota, the small sample sizes, the insufficient data, the absence of a control group, and the quality of the studies involved.

3. Results

3.1. Literature search

A total of 13205 articles were retrieved by using database searches like Scopus (3272), PubMed (4520), and Google Scholar (5413). The 11961 articles were retrieved after the initial scrutiny to identify duplicates and review studies. Later, 135 articles were left after a full text identification, removal of unhealthy population studies and those not meeting inclusion criteria, and till now, 13067 articles have been excluded. In the second pass of eligibility, these 100 articles were reviewed and finally, 24 articles were selected for this review (Fig. 1);

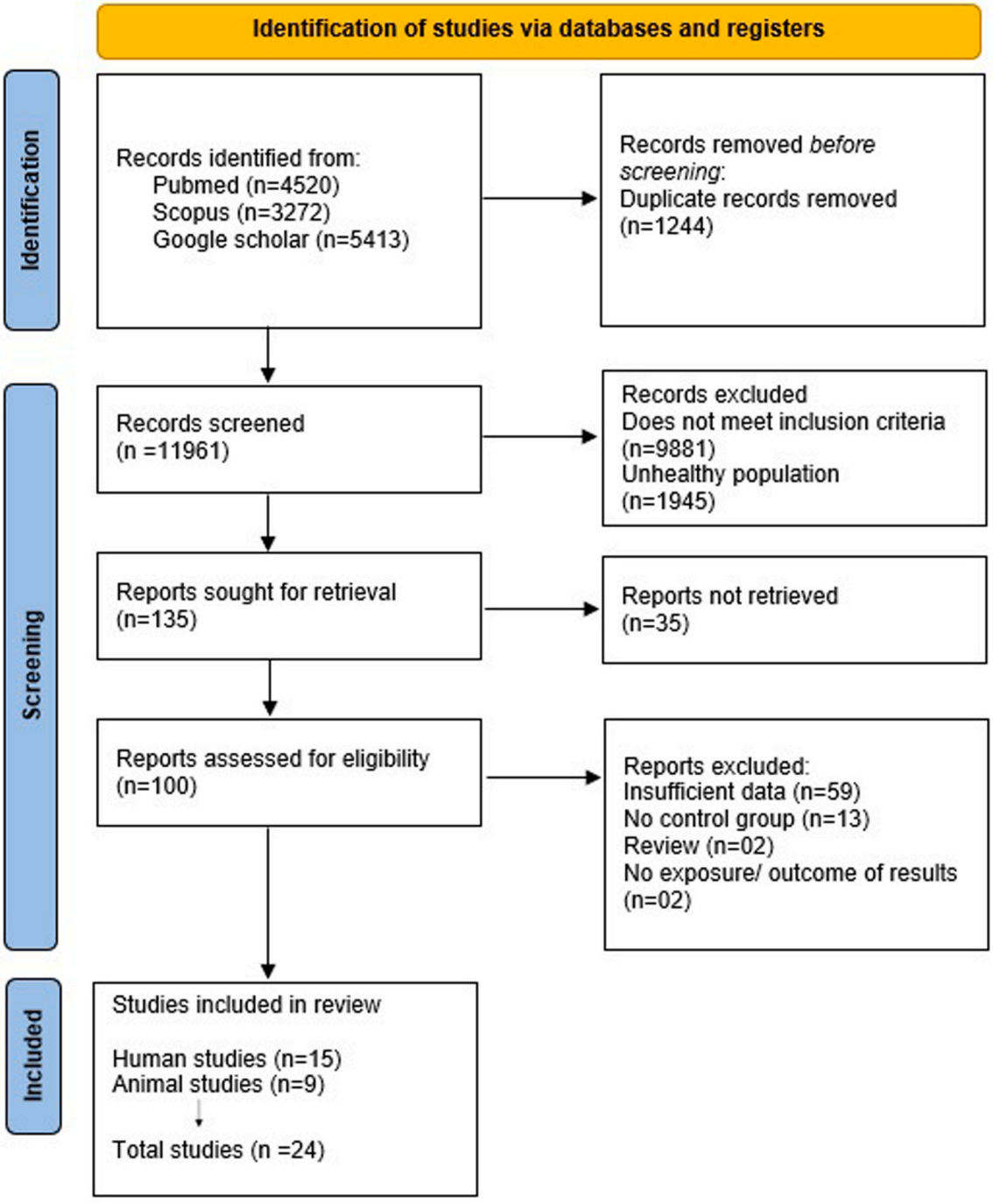


Fig. 1. Flow diagram following the PRISMA guidelines.

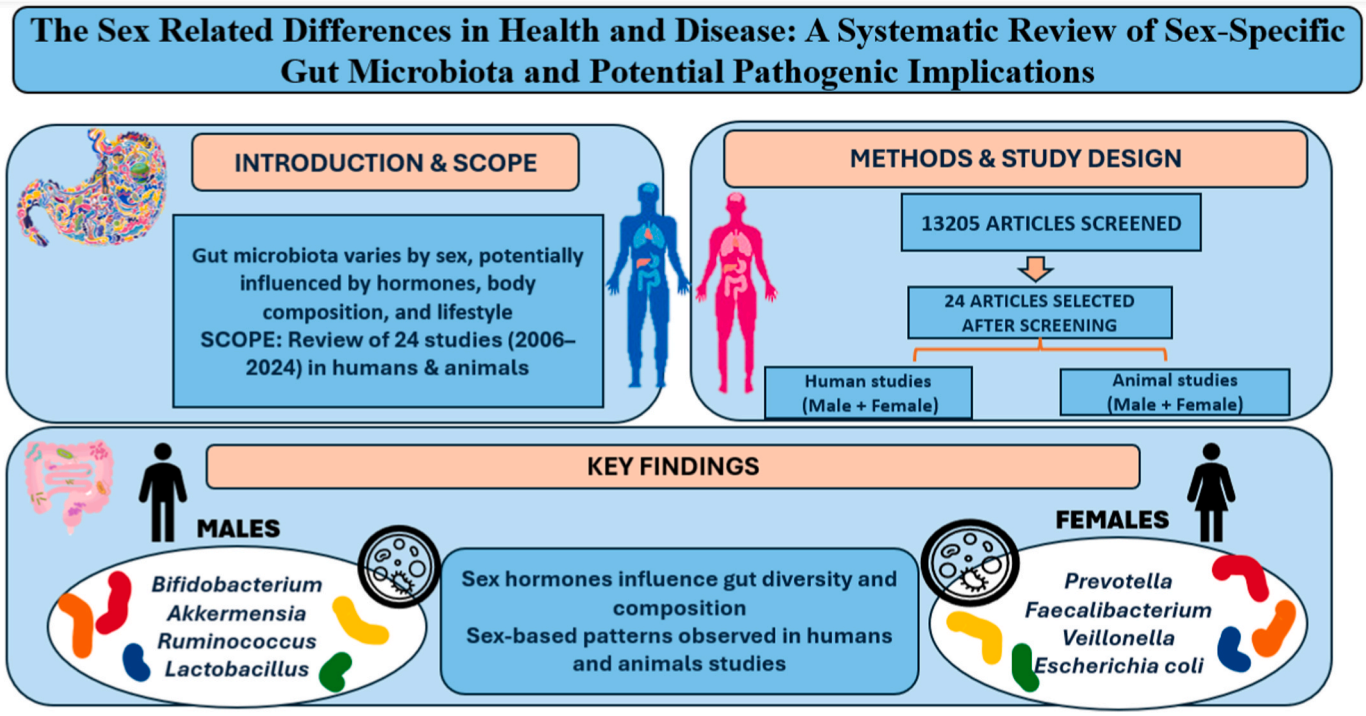


Fig. 2. Graphical illustration of the selection criteria for preparing the systematic review and the final analysis.

shown in Fig. 2; and the criteria of exclusion at this stage were 1) the study was not conducted in different sex oriented subjects, 2) sex differences did not fall into the same conditions, and 3) participants do not compile with the selection criteria; having gastrointestinal related diseases and unhealthy population.

3.2. Study characteristics

This systematic review encompassed 24 research articles, including 15 human studies [19–33], 09 animal studies [34–42]. The final 24 articles reported with characteristics in Tables 1 and 2. The twenty four selected studies were published between 2006 [19] and 2024 [33] and show a very stable trend, six articles were published in 2019 [27–30,39,40], three articles published in 2018 [25,26,38], three studies published in 2016 [23,24,36], two studies published in 2013 [34,35], three studies published in 2023 [31,41,42], two studies published in 2024 [32,33], two study published in 2014 [20,21], one study published in 2015 [22], one study published in 2006 [19] and one study published in 2017 [37], . Participant aged 1year [23] to 90 [23] years. All multi-strategy approaches are involved in this review. Most studies were conducted in China(n = 6) [20,26,32,39–41] and remaining in other countries including the USA (n = 7) [21–23,30,34,36,42], Netherlands (n = 3) [27,37,38], Spain (n = 1) [24], Japan (n = 1) [28], Italy (n = 1) [25], Isreal (n = 1) [33], Hungary (n = 1) [31], Germany (n = 1) [19], Canada (n = 1) [35], Korea (n = 1) [29]. In these studies, the data is classified into two groups; studies including humans (male and female) n = 15, studies including animals (male and female) n = 09. The mean age of participants is 53.5 years (range 18–89) (SD 16.2). range from 1 years [23] to a maximum 90 years [23].

As per the inclusion criteria, all studies utilizing NGS, including shotgun sequencing or 16S rRNA amplicon sequencing, and independent of culture-based techniques, are included. Twenty two studies used Illumina sequencing of the 16S rRNA gene [20–26,28–33,35–42], two studies used shotgun metagenomic sequencing [27,34], one study used 16S rRNA-targeted oligonucleotide probes/fluorescence *in situ* hybridization analysis [19].

The hypervariable regions used for analysis included V1-V2 (n = 2) [29,37], V3-V4 (n = 6) [22,26,28,31,32,39], V4 (n = 7) [24,30,33,34,40–42], V13 (n = 1) [21], V69 & V35 (n = 1) [21], V3-V5 (n = 1) [23] , and no specified region mentioned (n = 7) [19,20,25,27,35,36,38].

Alpha (α) diversity is a measure of the diversity within a subject, while beta (β) diversity compares the diversity between subjects [43]. Both Alpha and beta diversity were measured in six studies [25,26,30,33,38,42], while four studies investigated only the alpha diversity [24,29,32,34], and fourteen studies investigated only the beta diversity [19,20,21,22,23.27.28,31,35,36,37,39,40,41]. For the studies that investigated alpha diversity, the Shannon index was used in all of the studies; other indices used included the Chao1 index (n = 4) [25,28,29,39], richness (number of observed species) (n = 2) [29,38], Simpson's index (n = 4) [29,30,38,42], phylogenetic diversity (PD) (n = 1) [22], and orientation of Jensen-Shannon divergence (n = 1) [21]. Beta diversity was measured using UniFrac distance analysis (weighted and unweighted) (n = 09) [22,24,25,28,30,33,36,39,42], principal co-ordinates statistical analysis (PCoA) method (n = 11) [22,24,26,28,30–34,36,42], Bray-Curtis analyses (n = 6) [23,27,30,31,33,42], Jaccard index (n = 1) [27] and Simper index (n = 1) [23],.

The studies enclosed in the systematic review are divided into two groups, one group contains all the human studies (Table 1), and the other group includes all the animal studies (Table 2).

3.3. Sex differences in in overall microbial composition; alpha and beta diversity

3.3.1. Alpha and beta diversity in humans

Sex-based differences in gut microbial diversity were consistently reported across multiple human studies, highlighting distinct patterns in both alpha and beta diversity metrics. Alpha diversity, which reflects within-subject microbial richness and evenness, was generally higher in females, particularly in mucosa-associated microbiota, as demonstrated by Borgo et al. (2018) [25] and Sinha et al. (2019) [27]. However, some studies reported context-dependent trends. For instance, Dominianni et al. (2015) [22] found that among normal-weight individuals, males

Table 1
Characteristics of human Studies regarding sex differences in gut microbiota.

Author, Year, Country, Ref	Participant	Male-Female ratio M: F	Age	Study Method	Outcomes
Mueller,2006, Germany [19]	Total 230	N/A	Adult group (aged 20–50 years), Elderly group (>60 years of age)	14 Group and species-specific 16S rRNA-targeted oligonucleotide probes/fluorescence *in situ* hybridization analysis	At the species level, no sex differences were observed for *Bacteroides vulgatus* and *Bacteroides putredinis*. There was no detectable sex effect for any of the other microbial groups. ↑*Bacteroides-Prevotella* group in males than in females.
Feng, 2013, China [20]	Total 108 46 Male, 62 Female	45:55	42.8–51.0 years	16S rRNA gene sequencing	The fecal *F. prausnitzii* amount was not significantly different between obese and lean subjects. Men possessed a significantly lower level of fecal *F. prausnitzii* than women, and the significant and positive correlation of fecal *F. prausnitzii* quantity with fasting glucose level was observed in men, not in women
Ding & Schloss, 2014, USA [21]	Total 300 149 males, 151 females	50:50	18–40 years	16S rRNA gene sequencing	Sex was associated with community types (fecal, oral, vaginal, skin) identified in the stool. Males were 3 times more likely than females to harbor stool community type. Stool community in males had low levels of *Bacteroides* but higher *Prevotella.*
Dominianni, 2015, USA [22]	Total 82 51 males, 31 females	62:38	30–83 years	16S rRNA gene sequencing	Sex was significantly associated with the overall gut microbiome composition. ↓*Bacteroidetes* in females. The relationship between BMI and gut microbiota composition was significant in females, but not in males. ↓Shannon-diversity indices for overweight and obese subjects compared with normal-weight subjects in females, but no difference in males.
Singh & Manning, 2016, USA [23]	Total 200 141 male, 126 female	53:47	1–90 years	16S rRNA gene sequencing	Sex was significantly associated with the overall gut microbiota composition: At the genus level, ↑*Bacteroides* in females, ↑*Escherichia* in males. There was no sex difference in gut microbiota in healthy individuals. There was a significant difference between sexes in individuals with enteric infection: ↑*Enterobacteriaceae* among 11 different features in males, ↑*Bacteroidaceae* among 43 different features in females.
Haro, 2016, Spain [24]	Total 75	52:48	Females 60.31 ± 1.40, males 61.15 ± 1.27	16S rRNA gene sequencing/MiSeq (Illumina)	The microbiota diversity and overall community composition were not significantly different between sexes. ↑*Veillonella* and *Methanobrevibacter* at the genus level, *Bacteroides plebeius* and *Coprococcus catus* at the species level in males. ↑*Bilophila* at the genus level, *Bacteroides caccae* at the species level in females. There were no differences at the phylum level and in the F/B ratio between sexes when considered independently of the BMI. The F/B ratio was higher when BMI ≤33 kg/m^2 and lower when BMI >33 kg/m^2 in males compared with females. ↓*Bacteroides* at the genus level in males when BMI >33 kg/m^2.
Borgo, 2018, Italy [25]	Total 40	50:50	males 48.7 ± 10.2, females 51.7 ± 8.3,	16S rRNA gene sequencing/MiSeq (Illumina)	There was no difference in lumen-associated microbiota between the sexes. ↑α-diversity in MAM in females. ↑*Actinobacteria* and *Lactobacillales* in MAM of females. ↑*Bifidobacterium* spp. and *Streptococcaceae*. ↓*Veillonellaceae* and unclassified *Clostridia* in MAM of females.
Gao, 2018, China [26]	Total 551	47:53	males 37.8 ± 17.3, females 35.6 ± 14.3	16S rRNA gene sequencing	There was no significant overall difference in gut microbiota between the sexes. ↑α-diversity in females. Regardless of the BMI, there were no significant differences at the phylum, class, order, or family levels between the sexes, but ↑*Ruminococcus* at the genus level in females. In obese subjects, at the genus level, ↑*Bifidobacterium*, *Coprococcus*, and *Dialister* in females, ↓*Phascolarctobacterium* in females, ↑*Fusobacterium* in males. In underweight subjects, at the genus level, ↓*Sutterella* in males.

(*continued on next page*)

Table 1 (*continued*)

Author, Year, Country, Ref	Participant	Male-Female ratio M: F	Age	Study Method	Outcomes
Sinha, 2019, Netherlands [27]	Total 1135	42:58	18–81 years	Shotgun sequencing	Sex was significantly associated with the overall gut microbiota composition, 12 microbial species, and 43 metabolic pathways. ↑*Akkermansia muciniphila* in females. ↑Richness of antibiotic resistance genes in females.
Takagi, 2019, Japan [28]	Total 277	50:50	20–89 years	16S rRNA gene sequencing/MiSeq (Illumina)	There was no significant difference in α-diversity between males and females. ↑Genera *Prevotella*, *Megamonas*, *Fusobacterium*, and *Megasphaera* in males. ↑Genera *Bifidobacterium*, *Ruminococcus*, and *Akkermansia* in females.
Shin, 2019, Korea [29]	Total 57 31 males, 26 females	55:45	Male age 37.45 ± 1.91 Female age 46.15 ± 2.26	16S rRNA gene sequencing	Men and women in the High group harbored more diverse gut microbial communities than others. In men, the abundance of *Acinetobacter*, *Dorea*, *Ruminococcus*, and *Megamonas* correlated significantly with testosterone levels. Women in the High group have more *Bacteroidetes* and less *Firmicutes* phyla than those in the Low group. Genera *Slackia* and *Butyricimonas* were significantly correlated with estradiol levels
de la Cuesta-Zuluaga, 2019, USA [30]	Total 8924 Male female no not specified	NA	20–69 years	16S rRNA gene sequencing	Young adult women had consistently higher alpha diversity and predicted microbiota age than men in the US, UK, and Colombian cohorts. Specific taxa such as *Ruminococcaceae*, *Lachnospiraceae*, and *Akkermansia* were associated with higher diversity and enriched in females. These sex-based differences were not observed in the Chinese cohort, suggesting possible geographic or lifestyle influences on microbiome aging. Overall, the findings highlight age- and sex-dependent patterns in gut microbiome composition.
Kalabiska, 2023, Hungary [31]	Total 124 62 males, 62 females	50:50	16–20 years	16S rRNA gene sequencing	Predominance of *Streptococcus* and *Veillonella* genera was visually greater in females vs. males and in water polo players vs. controls. Compared to females, males had significantly higher relative abundance of the following genera: *Atopobium*, *Pravotella* very large effect size. Compared to females, males had significantly lower relative abundance of the genera: *Fusobacterium*, *Gemella*, and *Streptococcus*. Compared to the control group, water polo players had significantly higher relative abundance of the genus *Veillonella* and significantly lower relative abundance of the genus *Gemella*.
Ghazal, 2024, China [32]	Total 44 22 males, 22 females	51:49	Athlete group 21.55 ± 2.42 years Control group 21.27 ± 2.51 years	16S rRNA gene sequencing	Female Elite Athletes revealed notable components such as *Bacteroides* (28.48 %), *Prevotella* (21.86 %), *Megamonas* (11.43 %),*Megasphaera* (6.01 %), and *Lachnospira* (3.75 %). Conversely, male Elite Athletes exhibited a distinct microbial composition, with *Prevotella* (27.00 %), *Bacteroides* (15.82 %), *Megamonas* (11.55 %), *Faecalibacterium* (9.63 %), and *Alloprevotella* (5.77 %) as the primary genera. Differences in alpha and beta diversity measures were observed between male and female Elite Athletes, indicating variations in the overall diversity of their gut microbiota
Shalmon, 2024, Isreal [33]	Total 40 22 males, 18 females	55:45	Athlete group 43 ± 6.5 years, Control group 41 ± 7.4 years	16S rRNA gene sequencing	No difference in alpha diversity was observed between males and females in any of the groups. Female runners showed a lower abundance of *Enterococcus*, a higher abundance of *Lactobacillus*, and a higher abundance of *Methanosphaera* than male runners, while male runners exhibit lower abundance of *Enterobacteriaceace* & *Lactobacillus ruminis* while higher abundance of *methanosphaera*.

exhibited higher alpha diversity, whereas in overweight groups, females had greater diversity, suggesting a sex BMI interaction. Gao et al. (2018) [26] observed similar BMI-dependent sex effects, though the overall alpha diversity differences were not statistically significant. Conversely, Shin et al. (2019) [29] reported no significant sex-based differences in Shannon, Chao1, or Simpson's Evenness indices. In athletic populations, Ghazal et al. (2024) [32] found that male elite athletes exhibited higher alpha diversity than females, suggesting that physical training and sex may jointly shape microbial richness. Complementing these findings, beta diversity analyses used to assess between-subject variation in microbial composition and consistently revealed significant sex-specific clustering patterns. Studies by Ding et al. (2014) [21], Haro et al. (2016) [24], Dominianni et al. (2015) [22], and Takagi et al. (2019) [28] all reported distinct gut microbial community structures between males and females using PCoA plots based on UniFrac or Bray–Curtis dissimilarity metrics. Borgo et al. (2018) [25] specifically noted such sex-related compositional differences in mucosa-associated but not lumen-associated microbiota. Similarly, Ghazal et al. (2024) [32] and Kalabiska et al. (2023) [31] observed distinct clustering patterns in gut and oral microbiota, respectively, between male and female athletes, reinforcing the role of sex in shaping inter-individual microbial variation. In a large cross-sectional study of 1105 Colombian adults, de la

Table 2
Characteristics of animal Studies regarding sex differences in gut microbiota.

Author, Year, Country, Ref	Participant	Male Female ratio M: F	Age	Study method	Outcomes
Yurkovestskiv, 2013, USA [34]	Total 39 mice	50:50	4–13 weeks	Shotgun sequencing	The abundance of *Porphyromonadaceae, Veillonellaceae, Kineosporiaceae, Peptococcaceae, Enterobacteriaceae, Lactobacillaceae, Cytophagaceae, Peptostreptococcaceae, and Bacteroidaceae* at the family level was higher in the male mice than in the female mice
Markle, 2013, Canada [35]	Total 255 mice 123 males, 132 females	48:50	7–14 weeks	16S rRNA gene amplicon sequencing	Intestinal communities were distinct among male and female mice. Sex-specific differences in the intestinal community were most apparent in adult mice. Overall, the dominant genera in males were *Bilophila, Alistipes, Ruminococcus, Peptococcus*, and *Deltaproteobacteria*, whereas in females the dominant genera were *Lachnospiraceae*, *Lachno I.S.*, *Coprococcus 1*, and *Blautia.*
Org, 2016, USA [36]	Total 689 mice 341 females 348 males	49:51	8 weeks	16S rRNA gene sequencing	Phylum *Actinobacteria* (0.81 ± 0.19 males; 0.18 ± 0.1 females) and *Tenericutes* (1.08 ± 0.22 males; 0.35 ± 0.12 females) were more abundant in male than female mice. Genera with higher abundance in males as compared to female mice included *Allobaculum*, *Anaeroplasma*, and *Erwinia*, whereas *SMB53* from family *Colstridiaceae* and 3 members of family *Lachnospiraceae* (*Dorea, Coprococcus and Ruminococcus*) were more abundant in female mice
Fransen, 2017, Netherland [37]	Total 68 mice	49:51	7- to 10-week	16S rRNA gene sequencing	Conventional females had higher abundance of *Desulfovibrionaceae*, *Lactobacillaceae*, and *Verrucomicrobiaceae*, whereas conventional males had higher abundance of *Ruminococcaceae* and *Rikenellaceae*. Female recipients had a higher abundance of *Lactobacillaceae* and male recipients had a higher abundance of *Desulfovibrionaceae*, *Ruminococcaceae*, and *Porphyromonadaceae*. Four weeks after transfer, most differences were still dependent on the sexof the host, but there were also some donor-dependent differences. For example, lactobacilli were still more abundant in female recipients, whereas *Akkermansia* and *Prevotellaceae* were more abundant in male recipients. In these mice, *Rikenella*, *Lachnospiraceae*, and *Desulfovibrionaceae* were increased after transfer of male microbiota, but *Prevotellaceae* was increased after transfer of the female microbiota.
Elderman, 2018, Netherland [38]	Total 10 mice, 5 males 5 females	50:50	8 weeks	16S rRNA gene sequencing	*Lactobacillus plantarum* and *Bacteroides distasonis* were enriched in B6 females as compared to B6 males, while *Bifidobacterium* was enriched in BALB/c females as compared to BALB/c males. Overall, males show less diversity and richness as compared to females. Sex explained 11.6 % of the variance in microbiota composition
Zhao, 2019, China [39]	Total 20 deer 10 males 10 females	50:50	Juvenile 1–2-year Adults 4–10 years	16S rRNA gene sequencing	At the phylum level, the *Firmicutes* were most abundant in JMF (71.15 %) followed by AFF (58.66 %), JFF (55.90 %), and AMF (55.66 %). On the contrary, the *Proteobacteria* was abundant in AMF (28.67 %) followed by JFF (19.43 %), AFF (17.45), and JMF (10.35 %). The enrichment of *Bacteroidetes* was observed in AFF (20.65 %), JFF (19.17 %), JMF (17.02 %), and AMF (12.59 %). Amongst all groups, the presence of *Pseudomonas* was recorded as higher in AMF (12.90 %) and AFF (12.31 %) in average
He, 2019, China [40]	Total 293 pigs 108 female gilts, 185 male boars	58:42	80,120, 140 days	16S rRNA gene sequencing	*Veillonellaceae, Roseburia, Bulleidia*, and *Escherichia,* which showed the higher abundance in boars, and *Treponema* and *Bacteroides* which were over-represented in gilts. Castration significantly shifted the fecal microbiota composition of the boars toward that of gilts. At the family level, eight families were identified with significantly different abundances between entire boars and gilts in the Duroc population. *Veillonellaceae* and *Enterobacteriaceae* were more abundant in entire boars. However, *Spirochaetaceae* and *Bacteroidaceae* were significantly enriched in gilts. At the genus level, a total of 8 bacterial genera displayed differing enrichments between entire boars and gilts. For example, *Bulleidia* and *Escherichia* were of higher abundance in entire boars, whilst *Treponema* and *Bacteroides* were significantly enriched in gilts.
Gan, 2023, China [41]	Total 42 mice 18 female, 24 male	4:3	Age not defined	16S rRNA gene sequencing	*Firmicutes* (33.8 % for male and 49 % for female), *Bacteroidota* (46.3 % for male and 33.8 % for female), *Proteobacteria* (4.7 % for male and 8.8 % for female), and *Actinobacteriota* (3.2 % for male and 2.5 % for female) were the main enrichment of the microbial community, and the relative abundance of other bacteria was lower than 1 %.
Sisk-Hackworth, 2023, USA[42]	Total 77 mice 37 female, 40 male	52:48	10 weeks old.	16S rRNA gene sequencing	*Males had higher abundance of Alistipes and Rikenellaceae, while females showed enrichment of Lactobacillus and Erysipelotrichaceae*

Cuesta-Zuluaga et al. (2019) [30] found that women exhibited significantly greater alpha diversity than men, as measured by the Shannon index, even after adjusting for multiple covariates such as BMI, age, and lifestyle factors. Beta diversity analysis based on Bray–Curtis dissimilarities revealed significant sex-based differences in overall microbial community structure.

3.3.2. *Alpha and beta diversity in animals*

In animal studies, notable sex-specific differences in gut microbial diversity have been observed. Elderman et al. (2018) [38] reported that female mice displayed significantly higher alpha diversity and richness compared to males, with sex accounting for 11.6 % of the variance in microbial composition. Similarly, Fransen et al. (2017) [37] demonstrated persistent sex-dependent beta diversity patterns following fecal microbiota transplantation, with recipient sex influencing the overall microbial community structure even four weeks post-transfer. Markle et al. (2013) [35] also found that adult male and female mice exhibited distinct intestinal microbial profiles, reflecting beta diversity differences associated with sex. Org et al. (2016) [36] extended these findings by showing that sex significantly contributed to the variation in microbial community composition across a large cohort of mice, further supporting the presence of sex-driven beta diversity. In pigs, He et al. (2019)

Table 3
Comparison of gut microbial diversity in males and females in humans studies.

Reference	Microbial Diversity in Male	Microbial Diversity in Female
Muller 2006 [19]	↑ *Bacteroidetes* ↑*Prevotella*	↓*Bacteroidetes* ↓*Prevotella*
Feng 2013 [20]	↓*F.prausnitzzi*	↑*F.prausnitzzi*
Ding 2014 [21]	↓*Bacteroides* ↑*Prevotella*	↑*Bacteroides* ↓*Prevotella* ↓*Ruminococcaece*
Dominian 2015 [22]	↑*Bacteroidetes* ↓*Firmicutes*	↓*Bacteroidetes* ↑*Firmicutes* ↑*Actinobacteria*
Singh 2016 [23]	↑*Escherichia* ↑*Paraprevotella* ↓*Enterobacteriae* ↓*Fecalibacterium* ↓*Bacteroides*	↑*Bacteroides* ↑*Dialister* ↑*Lactobacillus* ↑*Lactobacilaceae* ↑*Comamonadaceae* ↑*Comamonas* ↑*Enterobacteriaea* ↑*Fecalibacterium*
Haro 2016 [24]	↑*Veillonella* ↑*Methanobrevibacter* ↑*Bacteroides plebius* ↑*coprococcus catus*	↑*Bilophila* ↑*Bacteroides caccae*
Gao 2018 [26]	↓*Ruminococcus*	↑*Ruminococcus*
Borgo 2018 [25]	↓*Actinobacteria* ↓*Lactobacilllus* ↓*Bifidobacterium* ↓*Streptococcaecae* ↑*Veillonellaceae* ↑*Clostridia* ↑*Gemmiger formicilis*	↑*Actinobacteria* ↑*Lactobacilllus* ↑*Bifidobacterium* ↑*Streptococcaecae* ↓*Veillonellaceae* ↓*Clostridia* ↑*Bifidobacterium adolescentis*
Sinha 2019 [27]	↓*Akkermansia muciniphila*	↑*Akkermansia muciniphila*
Takagi 2019 [28]	↑*Prevotella* ↑*Megamonas* ↑*Fusobacterium* ↑*Megasphera* ↓*Bifidobacterium* ↓*Ruminococcus* ↓*Akkermensia*	↑*Bifidobacterium* ↑*Ruminococcus* ↑*Akkermensia* ↓*Prevotella* ↓*Megamonas* ↓*Fusobacterium* ↓*Megasphera*
Shin 2019 [29]	↑*Actinobacteria* ↑*Dorea* ↑*Ruminococcus* ↑*Megamonas* ↓*Bacteroidetes* ↑*Firmicutes*	↑*Bacteroidetes* ↓*Firmicutes* ↓*Actinobacteria*
de la Cuesta-Zuluaga, 2019 [30]	↓*Ruminococcaceae* ↓*Lachnospiraceae* ↓*Akkermansia*	↑*Ruminococcaceae* ↑*Lachnospiraceae* ↑*Akkermansia*
Kalabiska 2023 [31]	↑*Atopobium* ↑*Pravotella* ↓*Veillonella* ↓*Fusobacteriuym* ↓*Streptococcus*	↑*Veillonella* ↑*Fusobacteriuym* ↑*Streptococcus* ↑*Gemella*
Ghazal 2024 [32]	↓*Bacteroides* ↑*Prevotella* ↑*Megamonas* ↓*Megasphera* ↓*Lachnospira* ↑*Fecalibacterium* ↑*Alloprevatella*	↑*Bacteroides* ↓*Prevotella* ↓*Megamonas* ↑*Megasphera* ↑*Lachnospira* ↓*Fecalibacterium* ↓*Alloprevatella*
Shalmon 2024 [33]	↓*Enterobacteriaceae* ↑*Methanosphaera* ↓*Lactobacillus ruminis*	↑*Methanosphaera* ↑*Mitsuokella* ↓*Enterococcus* ↑*Lactobacillus*

Table 4
Comparison of gut microbial diversity in males and females in animals studies.

References	Microbial diversity in Male	Microbial diversity in Femlaes
Yurkovestvik 2013 [34]	↑*Porphyromonadaceae* ↑*Veillonellaceae* ↑*Kineosporiaceae* ↑*Peptococcaceae* ↑*Enterobacteriaceae* ↑*Lactobacillaceae* ↑*Cytophagaceae* ↑*Cytophagaceae* ↑*Peptostreptococcace* ↑*Bacteroidaceae*	↓*Porphyromonadaceae* ↓*Veillonellaceae* ↓*Kineosporiaceae* ↓*Peptococcaceae* ↓*Enterobacteriaceae* ↓*Lactobacillaceae* ↓*Cytophagaceae* ↓*Cytophagaceae* ↓*Peptostreptococcace* ↓*Bacteroidaceae*
Markle 2013 [35]	↑*Bilophila* ↑*Alistipes* ↑*Ruminococcus* ↑*Peptococcus* ↑*Deltaproteobacteria*	↑*Lachnospiraceae* ↑*Lachno I.S.* ↑*Coprococcus 1* ↑*Blautia*
Org 2016 [36]	↑*Actinobacteria* ↑*Tenericutes* ↑*Allobaculum* ↑*Anaeroplasma* ↑*Erwinia*	↑*Closatridiaceae* ↑*Lachnospiraceae* ↑*Dorea* ↑*Coproccus* ↑*Ruminococcus*
Fransen 2017 [37]	↑*Ruminococcaceae* ↑*Alistipes* ↓*Lactobacillus* ↓*Akkermensia*	↑*Lactobacillus* ↑*Akkermensia* ↓*Ruminococcaceae* ↓*Alistipes*
Elderman 2018 [38]	↓*Lactobacillus plantarum* ↓*Bacteroides dustasonis* ↓*Bifidobacterium*	↑*Lactobacillus plantarum* ↑*Bacteroides dustasonis* ↑*Bifidobacterium*
Zhao 2019 [39]	Juvenile male: ↑*Firmicutes* ↑*Ruminococcaea* ↑*Dore* ↑*5-7NI5* *Adult male:* ↑*Proteobacteria*	Juvenile female: ↑*Bacteroidetes* ↑*Proteobacteria* ↑*Clostridiales* ↑*Bacteroidales* *Adult female:* ↑*Firmicutes* ↑*Bacteroidetes* ↑*Pseudomonas* ↑*Lachnospiraceae*
He 2019 [40]	↑*Veillonella* ↑*Roseburia* ↑*Bulleidia* ↑*Escheria* ↑*Enterrobacteriace*	↑*Bacteroides* ↑*Treponema* ↑*Spirochaetaceae* ↑*Bacteroidaceae*
Sisk-Hackworth, 2023 [42]	↑*Alistipes* ↑*Rikenellaceae,*	↑ *Lactobacillus* ↑ *Erysipelotrichaceae*
Gan 2023 [41]	↓Firmicutes ↑Bacteroidata ↓Proteobacteria ↑Actinobacteria ↓Bacteroides	↑Firmicutes ↓Bacteroidata ↑Proteobacteria ↓Actinobacteria ↑Bacteroides

[40] demonstrated both alpha and beta diversity differences between intact male boars and female gilts and showed that castration altered the microbial diversity of males to more closely resemble that of females. These findings consistently indicate that sex plays a significant role in shaping both the richness (alpha diversity) and overall structure (beta diversity) of the gut microbiome in mammalian animal models. In order to evaluate sex-based variations in microbial diversity, Sisk-Hackworth et al. (2023) [42] examined gut microbiota throughout intestinal habitats in wild-type and hypogonadal mice. The study discovered that sex and reproductive status had a region-specific impact on within-sample diversity, even when particular alpha diversity measurements were not provided. Significant sex-related clustering was seen in the ileum and cecum by beta diversity analysis, which also helped to differentiate between wild-type males and females. This suggests that the makeup of the gut microbial community is site-dependently influenced by both sex and hormonal status.

3.4. Sex-specific differences in individual microbial taxa

3.4.1. Individual microbial taxa in humans

Despite the growing interest in the role of gut microbiota on human health over the past few decades, there has been limited research on the influence of sex on the gut microbiota within human intestines. Few of the studies have been addressed here for the impact of sex differences in humans based on individual taxa in gut microbiota in Table 3 and all the

specific and dominant genera in the animal studies as a comparison between males and females are present in Table 4.

In 2006, Muller et al. conducted a study [19] carried out by four research centers in France, Germany, Italy, and Sweden found that variations associated with sex ($P = 0.036$) were observed in the *Bacteroides-Prevotella* group (Bac303) throughout the entire study cohort, showing that males had greater levels compared to females. However, two species-specific probes aimed at *Bacteroides vulgatus* and *Bacteroides putredinis* revealed no variations in their abundance between males and females. No effects related to sex were observed for the other microbial groups.

In 2014, Ding et al. conducted a study [21] and researchers examined a dataset of 16S rRNA gene sequences from the Human Microbiome Project (HMP) Consortium and found a correlation between sex and the types of microbial communities identified in stool samples. It was noted that males were three times as likely to exhibit community type D (stool), characterized by lower levels of *Bacteroides* and higher levels of *Prevotella* in stool while females exhibit higher levels of *bacteroides* and lower level of *prevotella & ruminococcaecae* in vaginal community.In a study conducted by Dominian et al., in 2015 [22], in the USA that primarily focused on a White population, the gut microbiota of women was found to have a lower presence of the phylum *Bacteroidetes* (5.5 %) while males had a higher percentage of *Bacteroidetes* (11 %). In normal-weight males, *Bacteroides* levels are elevated, while in normal-weight females, *Firmicutes* levels are increased. The Shannon diversity index shows greater values in males when compared to females.

Another study conducted by Borgo et al., in 2018 [25], carried out in Italy indicated that the microbiota associated with the lumen did not reveal any differences, while the microbiota linked to the mucosa differed between males and females. In females, the mucosa-associated microbiota exhibited a higher abundance of *Actinobacteria*, *Lactobacillales*, *Streptococcaceae*, and *Bifidobacterium*, along with a lower presence of *Veillonellaceae* and unclassified *Clostridia*. While males exhibit higher abundance of *Veillonellaceae* and unclassified *Clostridia*. At the species level, *Gemmiger formicilis* was linked to males, whereas *Bifidobacterium adolescentis* was associated with females. In a study conducted by Haro et al., 2016 [24], in Spain, there were no differences in microbiota diversity, general composition, phylum levels, and the *Firmicutes/Bacteroidetes* ratio when body mass index (BMI) was not taken into account. A more detailed taxonomic analysis revealed a greater presence of *Veillonella* and *Methanobrevibacter* in males, while *Bilophila* was more abundant in females. At the species level, females showed a higher abundance of *Bacteroides caccae*, whereas males had a greater abundance of *Bacteroides plebeius* and *Coprococcus catus*. The differences in microbiota between sexes became more pronounced when analyzed based on BMI groups in this study.Sinha et al., 2019 performed a study [27] involving a large group ($n = 1135$) from the Netherlands, differences in sex were linked to 12 microbial species and 43 metabolic pathways, with females exhibiting a greater abundance of *Akkermansia muciniphila*, even after accounting for various confounding factors like diet, lifestyle, and medication. Nevertheless, despite these findings, sex accounted for only 0.5 % of the overall variance in gut microbiota in that research.

Research conducted by Takagi et al., 2019 [28] in Asia revealed different findings compared to those from studies in the West. In a particular study from Japan, no notable difference in α-diversity was found between male and female participants. This study indicated that males had significantly elevated levels of *Prevotella*, *Megamonas*, *Fusobacterium*, and *Megasphaera*, while females exhibited higher levels of *Bifidobacterium*, *Ruminococcus*, and *Akkermansia*. A study conducted by Gao et al., 2018 in China [26] found that there were no significant differences in taxonomy between males and females overall. Nevertheless, at the genus level, *Ruminococcus* was found to be more prevalent in females. While a sex-based difference was identified at the genus level, it was also affected by BMI.

Shin et al., 2019 conducted a study [29] on sex based differences and found that at the phylum level, there were no distinct phyla recognized as significantly different between the two sex groups. The relative abundances of *Bacteroidetes* and *Firmicutes*, which constitute over 95 % of the human gut microbial community, did not show significant differences between males and females, and the ratios of *Firmicutes* to *Bacteroidetes* were also comparable. The diversity, richness, and evenness of the bacterial community were analyzed and compared between males and females. The diversity measured by the Shannon index showed women at 6.064 ± 0.166 and men at 5.959 ± 0.150, with a p-value of 0.778 indicating no significant difference. In terms of richness using the Chao1 index, women had a value of 6857 ± 308.7 while men had 6841 ± 335.8, with a p-value of 0.976 suggesting no significant variation. The evenness assessed by Simpson's Evenness index revealed women at 0.0071 ± 0.0009 and men at 0.0068 ± 0.0006, with a p-value of 0.693 indicating that there is no significant difference between the sexes.In a study by Feng et al., 2013 [20]; the data depict that males had a notably reduced level of fecal *F. prausnitzii* (2.8 %) compared to females (5.5 %). In another study by de la Cuesta-Zuluaga et al. (2019) [30] found that females had a higher relative abundance of *Akkermansia* and *Ruminococcaceae*, while males exhibited increased levels of *Prevotella* and *Bacteroides*.

The gut microbial diversity based on sex differences were also identified in athletes. Ghazal et al. (2024) [32] investigated that gut microbial diversity was higher in male athletes as compared to female athletes. Variations in alpha and beta diversity metrics were noted between male and female Elite Athletes, reflecting differences in the overall diversity of their gut microbiota. It is particularly noteworthy that female Elite Athletes exhibited a reduced level of diversity, which may be influenced by sex related factors. The dominant genera in female elite athletes were *Bacteroides*, *Megasphera* & *Lachnospira*, while the dominant genera in male elite athletes were *Prevotella*, *Fecalibacterium* & *Alloprevatella*. Shalmon et al., 2024 [33] investigated the gut microbiota at taxonomic level in elite runners, Male runners expressed a significantly lower abundance of *Enterobacteriaceae*, a significantly higher abundance of *Methanosphaera*, and a significantly lower abundance of *Lactobacillus-ruminis*. In contrast, female runners showed a significantly higher abundance of *Methanosphaera* and a significantly higher abundance of *Mitsuokella*. Compared to male runners, female runners showed a significantly lower abundance of *Enterococcus* but a significantly higher abundance of *Lactobacillus*, and a significantly higher abundance of *Methanosphaera*. Kalabiska et al., 2023 [31] investigated the differences in microbial diversity in water polo players, as compared to females; the males had a higher abundance of the *Atopobium* and *Pravotella* and a lower abundance of the *Fusobacterium*, *Gemella*, and *Streptococcus* genera. The predominance of *Streptococcus* and *Veillonella* genera was greater in females as compared to males. Compared to females, males had significantly higher relative abundance of the *Atopobium*, *Pravotella*. Compared to females, males had significantly lower relative abundance of the genera *Fusobacterium*, *Gemella*, and *Streptococcus*.

3.4.2. Individual microbial taxa in animals

Research conducted on animals, primarily mice, has demonstrated distinct differences in gut microbiota composition based on sex. Nevertheless, certain studies have not identified notable sex-related effects due to the interference caused by confounding variables like diet, age, and genetic background of the host. Overall all the specific and dominant genera in the animal studies as a comparison between males and females are present in Table 4.

In a study conducted by Yurkovestvik et al., 2013 [34], involving non-obese diabetic (NOD)/ShiLtJ mice revealed that the levels of *Porphyromonadaceae, Veillonellaceae, Kineosporiaceae, Peptococcaceae, Enterobacteriaceae, Lactobacillaceae, Cytophagaceae, Peptostreptococcaceae*, and *Bacteroidaceae* at the family classification were greater in male mice compared to female mice. In accordance with the findings, no notable sex differences in alpha-diversity were observed in 4-week-old (prepubescent) NOD mice, while significant differences were

identified in 13-week-old (post-pubescent) mice. Elderman et al., 2018 [38] conducted research involving two distinct strains of mice (BALB/c and B6), It was found that male mice exhibited lower microbial diversity and richness compared to female mice, while the strain of the mouse had no significant impact on this outcome. *Lactobacillus plantarum* and *Bacteroides distasonis* were enriched in B6 females as compared to B6 males, while *Bifidobacterium* was enriched in BALB/c females as compared to BALB/c males. Overall, males show less diversity and richness as compared to females. Sex explained 11.6 % of the variance in microbiota composition Conversely, the proportion of *Firmicutes* to *Bacteroidetes* was greater in the BALB/c strain, but this ratio was unaffected by the sex of the mice. Furthermore, sex played a role in affecting the expression patterns of genes associated with immune functions in the colon, in a manner dependent on the mouse strain.

In a study performed by Org et al., 2016 [36] the analyses of the gut microbiota from 89 distinct inbred mouse strains showed noticeable variations in microbial composition and diversity between male and female mice within each strain, with the most pronounced sex-specific differences found in the C57BL/6J and C3H/HeJ strains. A taxonomic assessment revealed that male mice exhibited higher levels of the phyla *Actinobacteria* and *Tenericutes* than female mice across the overall cohort. At the genus level, *Allobaculum*, *Anaeroplasma*, and *Erwinia* were found to be more prevalent in male mice, while female mice showed higher levels of SMB53 from the family *Clostridiaceae* and three genera from the family *Lachnospiraceae* (*Dorea, Coprococcus*, and *Ruminococcus*). When the entire cohort was analyzed collectively, the variation in microbiota composition differed in both magnitude and direction among the mouse strains, revealing no distinct patterns based on sex. These findings indicated that the influence of sex might vary depending on the genetic background of the hosts.

Fransen et al., 2017 [37] investigated the microbial diversity in male and female mice and later performed fecal microbiota transplant to look into the impact of these transfers on GF mice. Conventional females displayed a greater abundance of *Desulfovibrionaceae*, *Lactobacillaceae* (specifically *Lactobacillus* at the genus level), and *Verrucomicrobiaceae* (notably *Akkermansia* at the genus level), while conventional males showed a higher prevalence of *Ruminococcaceae* and *Rikenellaceae* (with *Alistipes* at the genus level). One week after the transfer to germ-free recipients, the significant effect of the recipient's sex became evident, as all notable differences were influenced by the host's sex. Female recipients exhibited a higher abundance of *Lactobacillaceae* (*Lactobacillus* at the genus level), whereas male recipients had elevated levels of *Desulfovibrionaceae*, *Ruminococcaceae*, and *Porphyromonadaceae* (with *Odoribacter* at the genus level). After four weeks of post-transfer, most differences remained tied to the host's sex, but there were also some variations dependent on the donor.

Gan et al., 2023 performed a study [41] in bamboo rats and observed that there were significantly more bacterial genera in female bamboo rats compared to their male counterparts. Additionally, the study found that certain bacterial genera in females, including *Bacteroides*, *Colidextribacter*, and *Oscillibacter*, were notably more prevalent than in males. Moreover, *Firmicutes*, *Proteobacteria* were higher in females, while in males, *Bacteroidota* and *Actinobacteria* were abundant. In the study by Sisk-Hackworth et al. (2023) [42], Wild-type females exhibited higher relative abundance of *Lactobacillus*, *Turicibacter*, and *Clostridium sensu stricto 1* in the ileum compared to males, while wild-type males showed enrichment of *Bacteroides* and *Akkermansia*. In the cecum, wild-type females had greater levels of *Akkermansia* and *Eubacterium coprostanoligenes group*, whereas males displayed higher abundance of *Muribaculaceae* and *Alistipes*.

Zhao et al., 2019 investigated in a study [39] in juvenile and adult deer to find the differences in microbial diversity and found that at the phylum level, the *Firmicutes* were most abundant in juvenile male forest musk deer (JMF) (71.15 %) followed by adult female forest musk deer (AFF) (58.66 %), juvenile female forest musk deer (JFF) (55.90 %), and adult male forest musk deer (AMF) (55.66 %). On the contrary, the *Proteobacteria* were abundant in AMF (28.67 %) followed by JFF (19.43 %), AFF (17.45 %), and JMF (10.35 %). The enrichment of *Bacteroidetes* was observed in AFF (20.65 %), JFF (19.17 %), JMF (17.02 %), and AMF (12.59 %). Amongst all groups, the presence of *Pseudomonas* was recorded as higher in AMF (12.90 %) and AFF (12.31 %) on average. Overall, the *Firmicutes* are higher in juvenile males and adult females. The *Bacteroidetes* were abundant in juvenile females and adult females. The alpha diversity index did not reveal any significant differences in bacterial diversity between males and females or across different age groups. The intestinal microbiota of FMD was primarily composed of three phyla: *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, regardless of sex or age. Adult males and juvenile females exhibited a higher prevalence of *Proteobacteria*. The composition of *Bacteroidetes* remained consistent across sex and age in FMD. Notably, the relative abundance of the genera *Clostridiales* and *Bacteroidales* was greater in juvenile FMD. In contrast, adult FMD showed higher proportions of *Pseudomonas* and *Lachnospiraceae*. Additionally, juvenile males displayed increased levels of *Ruminococcaceae*, *Dore*, and *5-7N15*.

He et al., 2019 performed a study [40] in pigs and noticed a clear impact of the host's sex on the composition of gut microbes and recognized several bacterial taxa that are biased towards either sex. These comprised *Veillonellaceae, Roseburia, Bulleidia*, and *Escherichia*, which exhibited greater abundance in boars, while *Treponema* and *Bacteroides* were more prevalent in gilts. The fecal microbiota's α-diversity in gilts was notably greater compared to that of entire boars when looking at both observed species and Shannon indices. *Veillonellaceae* and *Enterobacteriaceae* were found to be more prevalent in entire boars. In contrast, gilts showed a significant enrichment of *Spirochaetaceae* and *Bacteroidaceae*. At the genus level, eight different bacterial genera exhibited varying levels of enrichment between the entire boars and gilts. Markle et al., 2013 [35] performed a study in nonobese diabetic germ-free and pathogen-free mice, the intestinal microbiomes varied between male and female mice. The differences in intestinal communities based on sex were especially noticeable in adult mice. Overall, the dominant genera in males were *Bilophila*, *Alistipes*, *Ruminococcus*, *Peptococcus*, and *Deltaproteobacteria*, whereas in females the dominant genera were *Lachnospiraceae*, *Lachno I.S., Coprococcus 1*, and *Blautia*.

4. Discussion

This systematic review provides compelling evidence that sex plays an important role as a biological variable affecting the gut microbiota composition in both humans and animals. While associations with health and disease risk are increasingly reported, the underlying mechanisms linking sex-related microbial differences to pathogenic processes remain incompletely understood. Although previous studies have predominantly focused on other modulating factors such as diet, geography, age, and antibiotic use, this review sheds light on how biological sex, hormonal levels, and reproductive status can shape microbial ecosystems across species and developmental stages. However it is important to note that not all studies reported significant sex-related differences; in several cohorts, overall diversity and taxonomic profiles were comparable between males and females. These inconsistencies likely reflect the influence of confounding variables such as diet, BMI, lifestyle, and treatment conditions, which were not always controlled for across studies.

Alpha diversity, which encompasses both the richness and evenness of species, is often indicative of gut health and resilience to disease; therefore, these disparities could have significant physiological consequences. Differences in taxonomic abundance between the sexes were also apparent. In various human studies [26,27], females showed higher levels of genera such as *Bifidobacterium*, *Akkermansia*, *Ruminococcus*, and *Lactobacillus*. These genera are typically linked to gut barrier function, immune modulation, and metabolic well-being. In contrast, males frequently displayed elevated levels of *Prevotella*, *Faecalibacterium*, *Veillonella*, and *Escherichia*, taxa that are sometimes associated with

inflammation or dysbiosis, especially when present in high amounts [43]. *Akkermansia muciniphila* a mucin degrading bacterium associated with metabolic health was found to be significantly more prevalent in females across several studies. This may indicate a sex-hormone-mediated enhancement of the mucosal environment, which supports mucin-rich niches that favor these bacteria. In a different large-scale cohort study conducted by Falony [44], involving two well-characterized cohorts the Belgian Flemish Gut Flora Project (n = 1106) and the Dutch Lifelines-DEEP study (n = 1135), sex exhibited a 10th effect size among 69 factors with significant correlations to overall variations in the microbiome community. Following the analysis conducted by the Human Microbiome Project (HMP), many recent studies investigating sex-related differences in gut microbiota through 16S rRNA gene sequencing have surfaced. Overall, it seems that the composition of gut microbiota varies between males and females, with alpha diversity (particularly Chao and Shannon metrics) generally being higher in females. However, individual studies diverge significantly regarding differences in microbial taxa between the sexes. Sex hormones appear to play a crucial role in influencing microbial composition. Research indicates that hormonal fluctuations throughout different life stages such as puberty, menstruation, pregnancy, menopause, and conditions like PCOS can alter gut microbiota profiles. The idea of the sex based differences [45] captures this bidirectional interaction: while gut microbes can metabolize and regulate hormones (for example, via β-glucuronidase activity), these hormones also influence gut microbial communities. A growing body of evidence suggests that sex hormones play a fundamental role in shaping the gut microbiota, as demonstrated in both human and animal studies. In humans, conditions characterized by altered sex hormone level such as polycystic ovary syndrome (PCOS) and menopause are associated with distinct changes in gut microbial diversity and composition [46–48] In patients with PCOS, gut dysbiosis was characterized by diminished alpha diversity and an increased abundance of specific genera like *Kandleria* and *Catenibacterium*, which were positively correlated with serum androgen levels and negatively with estradiol, indicating that hormonal imbalances significantly affect microbial communities [46]. Similarly, postmenopausal women displayed a distinct microbial profile when compared to premenopausal women, featuring a shift toward *Bacteroidetes* and *Verrucomicrobia* and a reduction in *Actinobacteria* and *Firmicutes*, reflecting the decrease in estrogen [47] highlighted further how the composition of microbiota correlates with endogenous testosterone and estradiol levels. In men, elevated testosterone levels were associated with greater diversity and an increase in genera such as *Acinetobacter* and *Ruminococcus*. In women, higher levels of estradiol were positively correlated with *Slackia* and *Butyricimonas* [48]. This suggests that hormonal levels not only relate to broader phyla changes but also connect with specific genera.

Animal studies have supported and expanded upon the human findings, demonstrating sex-based differences in gut microbiota composition across various species In particular, research involving mice, pigs, and deer, uncovered microbial signatures linked to sex, often appearing after puberty and influenced by hormonal levels. In studies with mice, female subjects generally exhibited higher quantities of *Lactobacillus*, *Akkermansia*, and *Desulfovibrionaceae*, while male subjects showed increased levels of *Ruminococcaceae*, *Enterobacteriaceae*, and *Porphyromonadaceae* [36,37]. These observations indicate not only differences in microbial taxa but also functional variations, such as differences in the production of short-chain fatty acids (SCFAs) or the metabolism of bile acids. Research conducted on pigs [40] demonstrated that the castration of boars shifted their microbiota toward a profile more reminiscent of females, highlighting the influence of androgen activity. These results offer experimental evidence that the connection between sex hormones and microbiota is both causal and reversible, at least within animal models.

The sexual dimorphism observed in gut microbiota may influence susceptibility to diseases. For instance, germ-free mice colonized with male microbiota exhibited elevated testosterone levels and altered immune responses. This information could be relevant for understanding sex related differences in autoimmune conditions, metabolic disorders, and treatment responses. Beyond sex itself, factors like BMI, physical activity, and age can interact with sex to shape gut microbiota. Numerous studies have indicated that the differences in microbiota between males and females became more pronounced when analyzed by BMI. In the study by Haro et al. (2016) [24], males with elevated BMI showed notably lower levels of *Bacteroides*, a pattern not noted in females. In athletic populations, microbiota profiles also varied by sex. Ghazal et al. (2024) reported [32] that male elite athletes had greater microbial diversity and higher levels of *Prevotella*, while female athletes exhibited lower diversity and increased *Bacteroides* levels. Shalmon et al. (2024) identified [33] differences in *Lactobacillus* and *Enterococcus* between male and female runners, indicating that sex interacts with physical activity to influence gut microbial ecosystems. Further, Kalabiska et al., 2023 [31] identified differences in polo layers between male and female, indicating that females had more *streptococcus*, *Veillonella*, and *Fusobacterium* as compared to male polo players who depicted increased *Prevotella* and *Atopium* diversity.

Further, Cong et al., 2016 conducted a study [49] on male and female infants, female infants exhibited a greater prevalence of *Clostridiates* at the order level and a lower prevalence of *Enterobacteriales* compared to males during the initial 10 days of life. Subsequently, an indicator species analysis was conducted to pinpoint the genera that represented the differences in gut microbiota between male and female preterm infants. Among the various microbes classified at the genus level, the populations of *Pantoea* and *Campylobacter* (within the *Proteobacteria* phylum) were found to be significantly greater in males compared to females. In contrast, the prevalence of unidentified microbes and *Yersinia* (in the *Formicate* and *Proteobacteria* phyla) was significantly higher in females than in males during the period from birth to 30 days of life, which shows that the average α-diversity was greater in females (0.58 ± 0.22) than in males (0.48 ± 0.26) during the first 30 days of life, and *Firmicutes* level was higher in females and *proteobacteria* higher in males.

Animal models have provided stronger causal evidence for hormonal regulation of the gut microbiota. Markle et al. (2013) [35] showed that sex differences were observed in mice after the puberty and there were clear differences in the gut microbiota, depicting its dependence on sex harmones. Castration of male mice led to a microbial profile resembling that of females, while testosterone supplementation restored the male-associated microbiota. Similarly, Yurkovetskiy et al. (2013) [34] used non-obese diabetic (NOD) mice to demonstrate that androgens protect against autoimmune diabetes through sex hormone–dependent modulation of the gut microbiota. Org et al. (2016) [36] extended these findings by showing that gonadectomy alters microbial composition in both male and female mice, with hormone replacement restoring sex-specific microbiota patterns. These studies collectively confirm that sex hormones causally influence gut microbial ecology, supporting the observations from human cohorts.

In addition to sex hormones, several host physiological factors differ between males and females and may contribute to sex-specific gut microbiome profiles. These include differences in immune system function, gut permeability, bile acid composition and metabolism (regulated by the liver), as well as gastrointestinal transit time [50]. For instance, bile acids, which are known to shape microbial communities, are synthesized and regulated differently in males and females, potentially contributing to microbial variation [51]. These factors collectively highlight the multifactorial nature of sex influence on the gut microbiota beyond hormonal regulation alone.Some methodological discrepancies exist in the literature that may influence observed sex-specific differences in the gut microbiome. Variations in sequencing methods (16S rRNA versus shotgun metagenomics), targeted regions (V1–V9), sample origins (fecal versus mucosal), and population demographics restrict cross-comparison between studies. These technological caveats highlight the need for standardized approaches to accurately characterize sex differences and interpret findings across studies Moreover,

functional differences such as gene expression (metatranscriptomics) and metabolic output (metabolomics) are not captured by 16S-based analyses, potentially underestimating biologically meaningful sex-specific variation. Integrative multi-omics approaches, including shotgun metagenomics, metatranscriptomics, and metabolomics, are increasingly necessary to reveal subtle but important sex-based distinctions in microbial function, gene regulation, and metabolite production that remain undetectable through taxonomic profiling alone. Emerging evidence, such as from Sisk-Hackworth et al. (2024) [42], shows that sex- and hormone-dependent differences may vary significantly between these regions, underscoring the need for more spatially resolved sampling strategies. Future research incorporating mucosal biopsies or region-specific sampling will be essential to fully characterize sex-based microbiome variation throughout the gut. Most human microbiome studies to date rely on fecal 16S rRNA gene sequencing, which primarily reflects the luminal microbial community in the distal colon.

Additionally, while our review emphasizes biological sex differences, it is important to recognize that gender, a sociocultural construct encompassing identity, roles, and behaviors, may also influence gut microbiota composition. Gender-related factors such as diet, health behaviors, stress exposure, and access to healthcare can differ significantly between individuals identifying as men, women, or gender-diverse, potentially shaping the microbiome independently or in interaction with biological sex. Future microbiome research should aim to incorporate both sex and gender variables, as recommended in recent frameworks for inclusive biomedical research [52].

Furthermore many investigations had limited sample sizes, lacked longitudinal data, or failed to control confounding factors such as diet, medication, or hormone contraceptive usage. Additionally, differences in beta diversity, which compares microbial communities between individuals, were reported inconsistently. While some studies identified distinct sex-linked community patterns, others noted little or no differences, potentially due to insufficient statistical power or homogeneity within populations.

5. Conclusion

This review emphasizes that sex is a significant yet often overlooked factor affecting the composition of gut microbiota. While females typically show greater alpha diversity and unique microbial taxa compared to males, the biological mechanisms, particularly the influence of sex hormones, require further exploration. The gut microbiome is evidently influenced by hormonal levels, body composition, age, and physical activity, all of which vary by sex differences. As awareness of the microbiome's role in various diseases grows, personalized microbiome-targeted therapies must take sex into account as a crucial element. Future studies should focus on incorporating sex stratified analyses, employing consistent methodologies, and investigating mechanistic insights through longitudinal and interventional research. Understanding the intricacies of the sex related differences could lead to new possibilities for sex specific diagnostics, preventative measures, and treatment options for microbiota-related disorders.

This systematic review points out that differences in gut microbiota based on sex are substantial, biologically significant, and influenced by multiple factors. Evidence from both human and animal research consistently shows that male and female hosts possess distinct gut microbial communities, with noticeable differences in alpha and beta diversity, taxonomic structures, and metabolic functions. These variations arise from genetic predispositions, lifestyle habits, and sex hormones like estrogen and testosterone, which seem to affect microbial colonization, diversity, and functional characteristics. The interaction between gut microbiota and sex hormones is both dynamic and reciprocal: hormones shape the microbiota, and microbial metabolites can influence hormone metabolism in return. This relationship is particularly pronounced during hormonal shifts such as puberty, pregnancy, menopause, and conditions like PCOS, all of which significantly alter microbial composition. Additionally, new studies suggest that the gut microbiome may play a role in sex specific vulnerability to various diseases, including metabolic disorders, immune dysfunction, and gastrointestinal issues. Despite these evident patterns, variability in methodologies, such as differences in sequencing technologies, data analysis methods, sample types, and population demographics, makes it challenging to generalize findings. Moreover, a few studies have directly explored mucosa-associated microbiota (MAM), which may have an even closer relationship with host microbe interactions compared to lumen-associated communities. To advance the field, future research should prioritize.

- Development of longitudinal and intervention-based studies that evaluate changes in microbiota throughout hormonal cycles and different stages of life.
- Implementation of standardized methods for microbiota analysis to ensure uniformity across various research studies.
- An increased focus on sampling from mucosal tissues rather than solely relying on fecal samples, to deliver a more comprehensive understanding of host-microbiota interactions.
- Investigative studies that examine how metabolites produced by microbes engage with hormonal receptors and influence signaling pathways.
- Clinical research that includes sex as a biological factor, particularly in microbiota-focused treatments like probiotics, prebiotics, and fecal microbiota transplantation (FMT).

Ultimately, a better understanding of the sex microbiota axis will not only refine our comprehension of host physiology and disease, but also pave the way for personalized, sex related sensitive approaches to health, disease prevention, and treatment. As microbiome science moves closer to clinical translation, acknowledging and incorporating sex-based biological diversity is not just beneficial it is essential. Nevertheless, it must also be recognized that several studies report no significant sex-based differences, and that factors such as diet, medication, BMI, and lifestyle may confound results; future studies should therefore carefully control for these influences to avoid misleading conclusions. Future research should therefore carefully control for these influences, while also pursuing mechanistic and longitudinal studies to confirm the preliminary links between sex-related microbial variation and disease pathogenesis.

CRediT authorship contribution statement

Tehreema Ghaffar: Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Federica Valeriani:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization. **Vincenzo Romano Spica:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

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.

Data availability

No data was used for the research described in the article.

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ABBREVIATIONS:

GI: Gastrointestinal Tract
LAM: Lumen-associated microbiota
MAM: Mucosa-associated microbiota
GF: Germ free
IBS: Irritable bowel syndrome
PRISMA: Preferred reporting items for systematic reviews and meta-analyses
NGS: Next-generation sequencing
DGGE: Denaturing gradient gel electrophoresis
PD: Phylogenetic diversity
PCoA: Principal coordinate statistical analysis
PCOS: Polycystic ovarian syndrome
BMI: Body mass index
HMP: Human microbiome project
NOD: Non obese diabetic
JMF: Juvenile male forest musk deer
AFF: Adult female forest musk deer
JFF: Juvenile female forest musk deer
AMF: Adult male forest musk deer
FMD: Forest musk deer
SCFA: Short-chain fatty acids

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Narrative Review

A review of the impact of intermittent ramadan fasting on wellbeing, nutrition and physical performance in different sports

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SUMMARY

Background: Athletes in different sports may experience a change in the physical performance during Ramadan Intermittent Fasting (RIF). The impacts of fasting on performance, coping behaviour and fatigue in athletes have not been fully researched.
Objective: To examines the effects of Ramadan Intermittent Fasting (RIF) on the physical performance of athletes in different types of sports. The approaches used to assess effects of intermittent fasting among young athletes have not yet been systematically reviewed.
Method: Following PRISMA guidelines, 3 independent searches on different databases to identify all the articles describing effects of RIF on physical performance of athletes with all available articles from 2014 up to April 2024.
Results: Among 422 articles identified, 31 studies met eligibility criteria. Fourteen studies reported the effect of Ramadan fasting on individual sports, three studies on combat sports, and fourteen studies on team sports.
Conclusion: The RIF negatively effects the physical performance, players coping behavior, nutritional status and fatigue capacities in different sports, although, the negative effects are greater for Handball, Basketball, Volleyball, Football and Swimming although the impact is less negative for Tennis, Badminton, Martial arts and Endurance sports. The athletes who maintain their total energy and macronutrient intake, training load, body composition, sleep length and quality are unlikely to suffer any substantial decrements in performance during Ramadan. Adding other recovery methods could improve performance and reduce the fatigue and muscle soreness experienced with increased game frequency during RIF.
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RESEARCH SNAPSHOT

Research Question: What does the literature reveal about the association between Ramadan intermittent fasting and its effects on the physical performance of athletes in different sports categories?

Key Findings: A systematic review of 31 studies that included athletes performing Ramadan intermittent fasting in individual sports, combat sports and team sports, findings are inconsistent across the included studies as the heterogeneity in results depends on the sports category, carbohydrate intake, sleep time, training session duration and recovery methods. Overall results depict negative impact of RIF in team sports as compared to individual and combat sports. The validity of RIF in association with performance in athletes requires further research.

1. Introduction

Ramadan is a holy and religious month for Muslims, in which they refrain from taking any liquid and food from sunrise to sunset. The duration of this holy Ramadan month can vary from 29 or 30 days as the length depends on the crescent moon sightseeing [1]. According to Gregorian calendar the Ramadan extends 11 days

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forward each year, so it may come in summer or in winter season [2]. Before the dawn Muslims eat food known as “Sahour” and after the suhoor they do not eat or drink anything until the sunset, and then after sunset they start eating and drinking known as “Iftar”. It is allowed to eat and drink between Suhoor and Iftar time [3]. In recent decades, the participation of Muslim athletes in major international sporting events has steadily increased. At the 1984 Los Angeles Olympics, Muslim representation was relatively limited, but by the 2012 London Olympics, approximately 3000 Muslim athletes competed, reflecting the growing presence of Muslim sports professionals at the global level [4,5]. As this trend continues, the impact of Ramadan fasting on athletic performance has become an important topic in sports science. Key events such as the 2014 FIFA World Cup, the 2016 Rio Olympics, and the 2018 Mediterranean Games all took place during Ramadan, raising concerns about how fasting affects athletes' endurance, hydration, and recovery. Some research suggests that fasting can lead to a decline in aerobic capacity and neuromuscular function, while other studies indicate that well-structured training and nutrition strategies can help mitigate these effects [4].

Given this increasing global participation of Muslim athletes, understanding the physiological and psychological impact of Ramadan fasting on performance is crucial. This review aims to analyze how intermittent fasting during Ramadan affects different sports and what strategies athletes and coaches can adopt to optimize performance while respecting religious practices [5]. The schedule of the major Olympic games and other major competitive events is not planned according to the Muslim calendar and to overlook this fact the Muslims players need to train their body to compete progressively even being in state of RIF. So, the abstain from eating and drinking during the training periods and competitive events have significant impact on the physical performance in sports [6].

Ramadan intermittent fasting (RIF) has been demonstrated to cause changes in energy metabolism, variations in hormone levels, and disturbances in sleep patterns, all of which may have an impact on sports performance. Rest during Islamic fasting helps conserve energy and optimize performance by reducing metabolic demands. It lowers cortisol levels, minimizing stress and muscle breakdown, while enhancing glycogen preservation for sustained endurance. Rest also supports cognitive function by preventing fatigue-related declines in focus and reaction time [3,6].

In recent years there has been an increased interest in studying the impact of RIF on physical performance in different sports activities. As the RIF is a fasting condition and during this state the glucose level in the body decreased which leads to glycogen depletion and protein degradation in the muscles which may leads to decrease in dribbling speed, aerobic capacity, speed, agility and speed endurance performance. Also, there is a decrease in weight reduction which is quite stressful in some sports [7]. Zarrouk et al., conducted a study of football player during RIF and controlled sleeping time, food intake and the training load and as a result there was no stress on the neuromuscular performance and there was no impact on the anthropometric parameters [8].

Some competitions and events and even training programs engage the players in sessions of more than 1 h and during RIF the dehydration could lead to decrease the performance and makes the recovery slow [9]. The dehydration in players is a possible risk factor for the decreased concentration ability, increased heart rate, increased perception of fatigue and reduced alertness [10,11]. The significant impacts of RIF on players can be managed if the changes are made in the training volume, dietary plan, increasing fluid intake and optimizing training program. The training periods during RIF are very significant and to make it better the decision of reducing training load and to manage it in the evening are good options for players [12]. Rebai et al., performed a study in which during RIF the two weeks of taper training (reduction in training volume) was observed in players, and which decreased the fatigue in players and help to maintain the same performance in competition as done before Ramadan [13].

Chaouachi et al., found in a study that during and after Ramadan there were no differences were observed for 30 s repeated jumps, squat jump, countermovement jumps and total fatigue score [14]. Souissi et al., observed that in Wingate test in players no difference of muscle power observed before and during RIF [15]. Fashi et al., observed in players no differences on short term maximal power for performing 5-min shuttle run test [16]. In some cases, Zerguini et al., observed that performance for 20-min shuttle tests decreased two weeks after the RIF [17]. Evermore, Kirkendall et al., found an increase in the scores of vertical jump and agility tests after the Ramadan month and it depicts the better performance [18]. Chamari et al., found an increment in performance of cyclist, being more accurate, tested by using visual information processing after two weeks of RIF compared to before RIF [19].

It has been suggested for a long time that the changes brought on by intermittent fasting during Ramadan may necessitate a reduction in the training load undertaken by Muslim athletes. A significant reduction in training load permits athletes to reduce the fatigue caused by intense training and enhance their performance in competition. It is crucial, therefore, to investigate whether athletes become more fatigued with during Ramadan and to take precautions in the event of a negative outcome.

1.1. Aim

The aim of this review paper is to review different studies including different type of sports including soccer, football, karate, runners, endurance and so many other sports performed during the Ramadan fasting and explore the changes in physical performance capacity in athletes performing before the Ramadan fasting, during Ramadan fasting and after the Ramadan fasting. Furthermore, this review is first of its kind as it discusses the effect of Ramadan intermittent fasting on most played sports and their effects of physical performance of athletes, otherwise before all the review papers discuss only few sports activities regarding the Ramadan intermittent fasting.

2. Material and methods

2.1. Search strategy

This review involved a search of the PubMed, Google Scholar and Scopus data sources for all articles published in English language published between 2014 and 2024, investigating the effect of Ramadan fasting on physical performance of athletes. The selection of studies from 2014 onward was based on the emergence of new research methodologies and advancements in sports science, particularly in understanding the effects of Ramadan intermittent fasting (RIF) on athletic performance. This timeframe ensures the inclusion of the most recent and relevant findings, reflecting contemporary training practices, dietary strategies, and physiological assessment techniques. Particularly, the review question was framed using PICOS framework and the following eligibility criteria were used: (a) Population: athletes; (b) Intervention: Ramadan fasting; (c) Comparison: age- and condition-matched control group; (d) Outcome: physical performance and health; Study: All studies. The search terms that were used (either combined or separated) were as follows: “TITLE-ABS-KEY ((Ramadan AND fasting) AND (physical AND activity OR sports OR athletic AND activity)) AND PUBYEAR >2014 AND PUBYEAR <2025 AND (LIMIT-

TO (LANGUAGE, "English")). Only those studies that concentrated on the impact of Ramadan fasting on different types of athletes were included. We excluded the studies that explored the impact of fasting on non-athletes' active healthy populations.

2.2. Data analysis

After reviewing the title and abstract of the eligible studies, we classified the articles into the following three categories (Table 1). Accordingly, we present the results of the eligible studies based on each category.

2.3. Inclusion and exclusion criteria

The inclusion criteria for this review were: 1. Study Design: Randomized studies published from 2013 to 2024, designed to evaluate the impact of Ramadan intermittent fasting (RIF) on nutrition and physical performance. 2. Population: Studies investigating the athlete population in developed and Muslim countries. All subjects included in the studies were healthy, ranging from 17 to 40 years of age [20]. 3. Scope: Studies that specifically examined the effects of Ramadan intermittent fasting on different sports activities, considering its impact on fatigue, agility, performance, and VO_2 max. 4. Outcome Measures: Studies were required to report changes in at least one of the following: a. Nutritional changes, including alterations in BMI, anthropometric measurements, biochemical markers, and dietary consumption data. b. Physical performance variations and behavioral adaptations regarding sport strategies. 4. Source Selection: To ensure a comprehensive and rigorous analysis, the review prioritized: a. Peer-reviewed journal articles to enhance reliability and validity. b. Studies providing an updated overview of the last ten years of research regarding the evolution of religious practices during Ramadan. Studies were excluded if they examined the impact of intermittent fasting on non-athletes or sedentary individuals, as the type of sports activity was a key factor in the analysis.

2.4. Selection process

All the results obtained were exported to the end note library for the selection process, where the first author reviewed the articles based on title and abstract reading to remove the duplicates. In addition, the first author screened all remaining titles and abstracts by looking at the inclusion criteria. Ineligible articles were removed and their reason for exclusion (not a study; not the correct population of athletes; not an intermittent Ramadan fasting strategy; or not the correct outcome measures) was noted.

3. Results

A total of 422 articles were retrieved by using database searches like Scopus, PubMed and Google scholar. The 92 articles were retrieved after the initial scrutiny to identify duplicates and review studies. Later 57 articles left after a full text identification and till now 365 articles have been excluded (Fig. 1). In the second pass of eligibility theses 57 articles reviewed and finally 31 articles selected for this review [20–50] and the criteria of exclusion at this stage 1) study was conducted on professional athletes 2) proper nutritional strategy of fasting not encountered 3) not compliance with rules of intermittent Ramadan fasting. 31 articles reported on different sports (Table 1). Participant numbers ranged from 8 [29] to 117 [28] and included adolescents aged 17 [20] to 40 [20] years. All multi-strategy approaches are involved in this review. Most studies were conducted in Muslim countries and remaining in other European countries including Tunisia (n = 7) [22,26,29,31,33,34,38], Singapore (n = 3) [41,45,49], Italy (n = 3) [40,42,44], United States (n = 3) [23,25,50], Algeria (n = 2) [27,35], Germany (n = 2) [36,46], Lebanon (n = 1) [20], Spain (n = 1) [16], Greece (n = 1) [24], Malaysia (n = 1)[30], Yemen (n = 1) [47], United Kingdom (n = 1) [48], Iran (n = 1) [37], Morocco (n = 1) [28], Kazakhstan (n = 1) [43], Portugal (n = 1) [39] and Turkey (n = 1) [32]. In these studies different types of sports activities were used including: Endurance Training [20–28], Karate [29], Archery [30], Judo [31], Kickboxing [32], Handball [33,34,36], Basketball [35,38,39], Volleyball [37], Tennis [40,42], Badminton [41], Soccer [43,46,48,49], Football [44,45,47] and Swimming [50]. These activities have been classified into 3 main groups: Individual Sports (n = 14) [20–28,30,40–42,50], Combat Sports (n = 3) [29,31,32], and Team Sports (n = 14) [33–39,43–49].

3.1. Analysis of yearly publications trend

The web of science data base includes 201 records on Ramadan fasting for sports. In Fig. 2 the line indicates the publications trends in the last ten years from 2014 until May 2024, showing the number of papers which are published each year (Fig. 2). Based on the observations by the data shown in Fig. 2, it is evident that this trend starts increasing in 2014 and till then 2024 its impact is increasing. It is noted that the data shown for 2024 is from January till May, so the upward trend will persist throughout the year. Although, from 2014 to 2024, the development showed an upward trend which dominantly indicating the increment in the research trends concerning the Effects of Ramadan fasting in sports activities. Moreover, the notable increment in these domains indicates that emphasis in this domain is notable.

3.2. Analysis of keywords

To determine the frontier and trending topics in the domain of Ramadan fasting in sports, we examined the frequency of keywords using the web of science database. Fig. 3 shows the frequency of keywords. This analysis depicts a list of 2644 research keywords, which provides an in-depth insight in this field. The most prominent keywords include "Human," "Physical activity", "Ramadan fasting," and "Fasting,".

3.3. Analysis of countries

Using Microsoft word, a country map (Fig. 4) was generated to find the leading countries in the research domain of Ramadan fasting in sports. Among the records which were analyzed, the publications originated from 450 countries. The top 10 countries in

Table 1
Types of sports categories in studies included in Review.

Type of Sport	Sports Categories	Studies [ref]
Endurance training, archery, tennis, badminton, swimming	Individual sports	[20–28,30,40–42,50]
Karate, judo, kickboxing	Combat sports	[29,31,32]
Handball, basketball, volleyball, football, soccer	Team sports	[33–39,43–49]

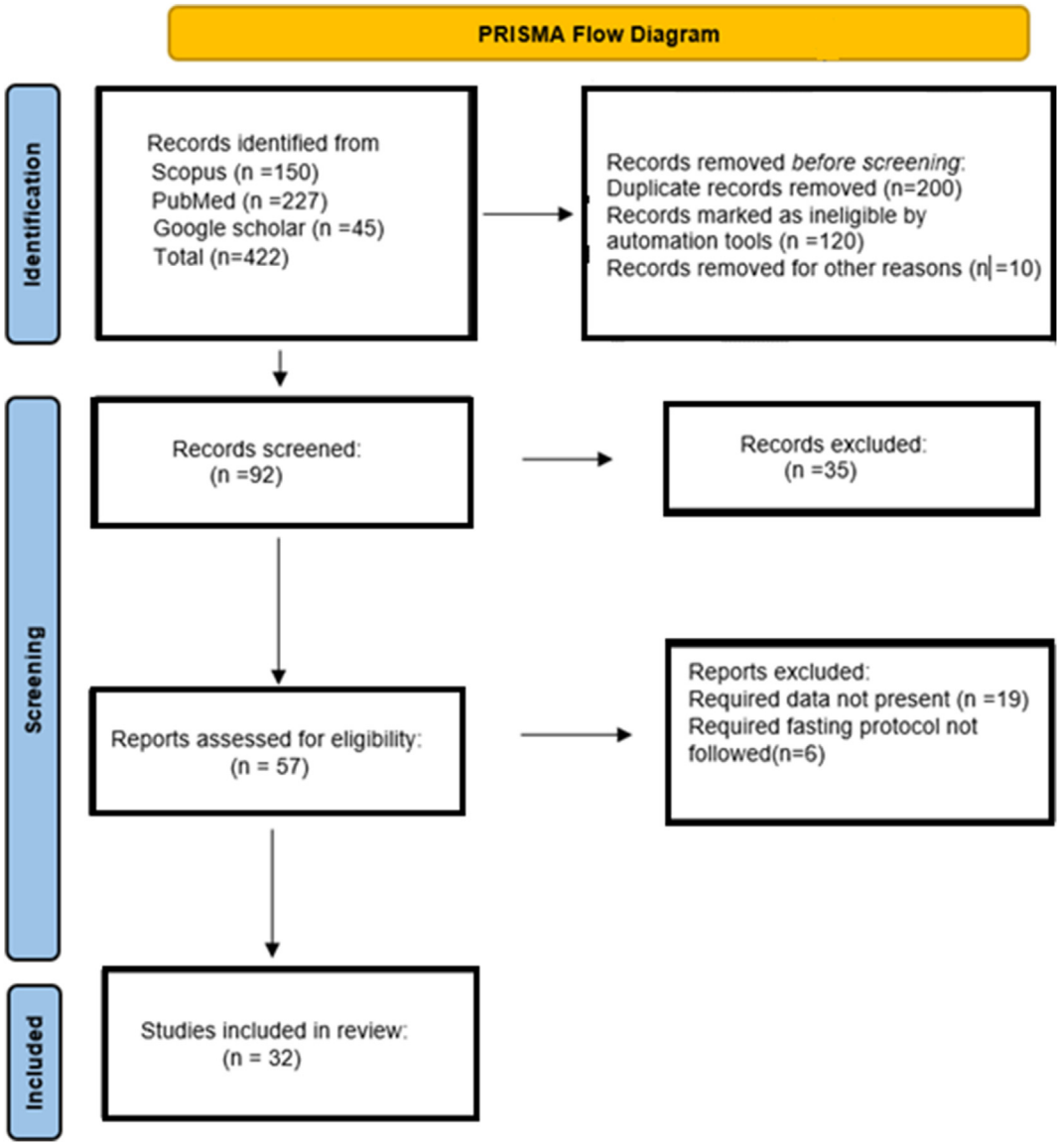


Fig. 1. Prisma flow diagram.

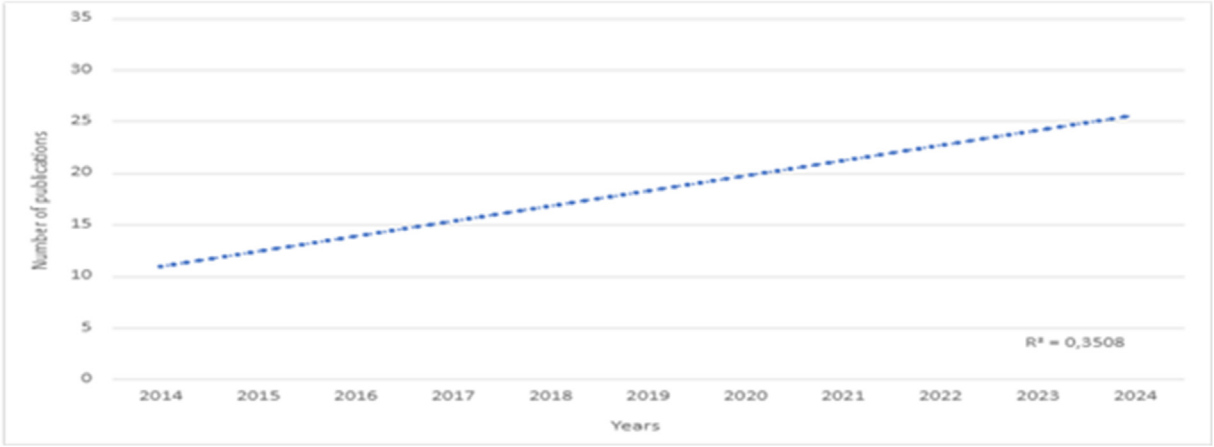


Fig. 2. Yearly publication trend. The number of publications about Ramadan fasting in sports increases in the last ten years.

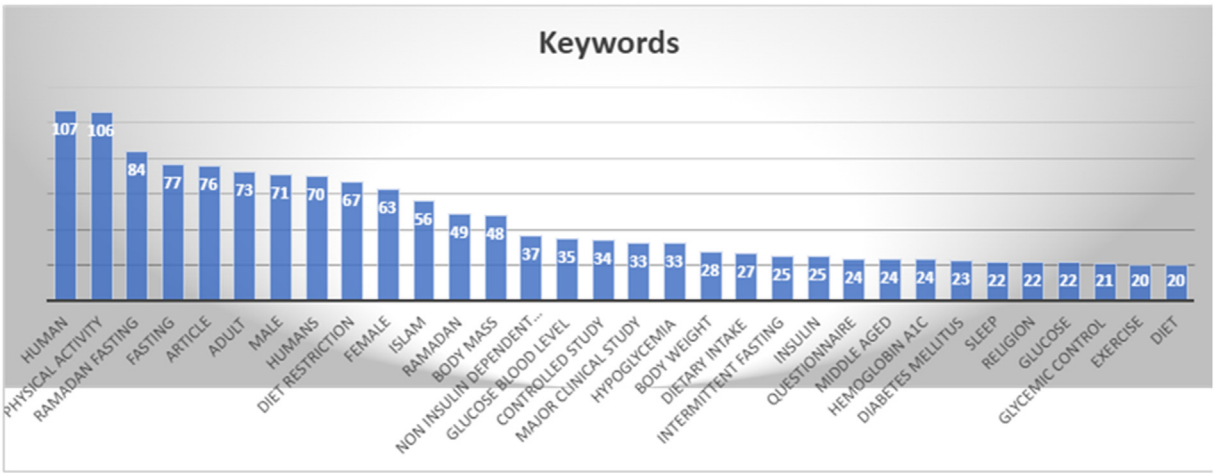


Fig. 3. Keyword trend. The number of Keywords about Ramadan fasting, and Physical activity shows highest trend.

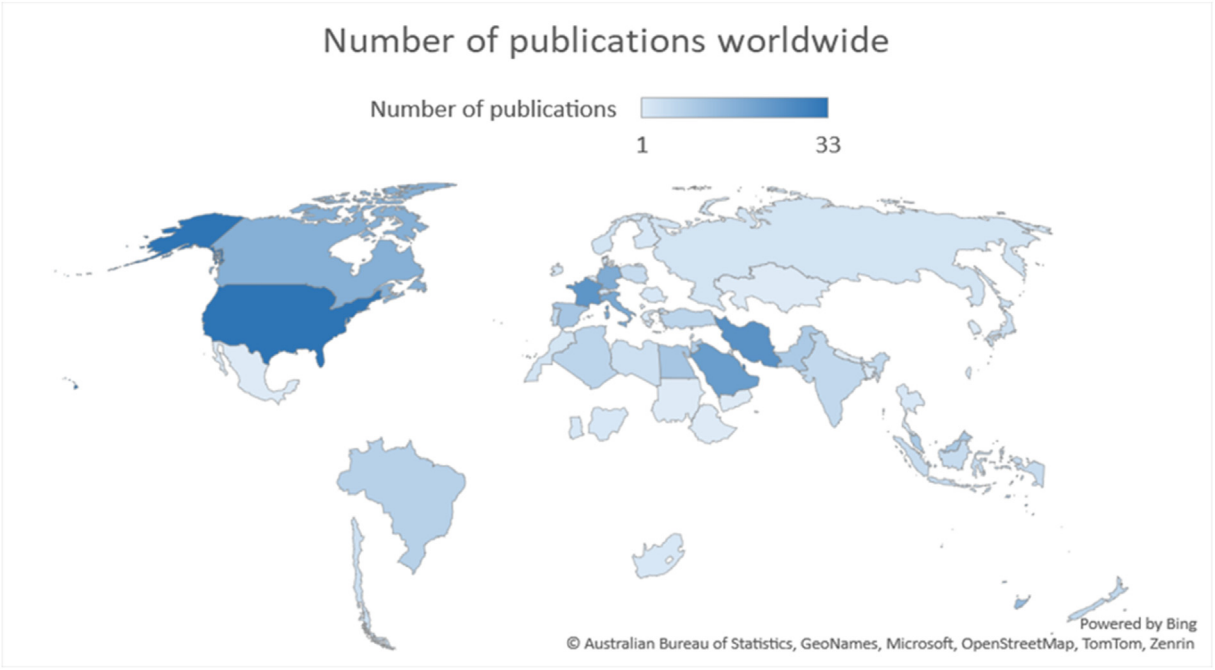


Fig. 4. Publications trend in worldwide. The publication trend worldwide is highest in the USA.

terms of publication count include USA, Qatar, England, Iran, France, Saudi Arabia, Arab Emirates, Italy, Germany and Canada. The USA emerged as the foremost contributor. The second and third position carried by Qatar and England. With passing years, Ramadan fasting in sports emerged with global identification, especially in the developed and Islamic countries.

In this paper the characteristics of original studies and main results are described in the form of a table (Table 2), including all the details of sports, origin, characteristics of participants, time frame of Ramadan Intermittent fasting and the categories of tests used to access the physical performance.

4. Discussion

4.1. Ramadan fasting in individual sports

The increased participation of Muslim athletes in international sporting events has led to a growing body of research exploring how fasting impacts competitive performance. Elite-level competitions such as the Olympics and FIFA World Cup often do not adjust their schedules to accommodate religious fasting, requiring athletes to adapt their training and nutrition strategies accordingly [9].

The physiological effects of Ramadan fasting vary depending on factors such as sport type, individual fitness levels, and adaptation strategies. Studies indicate that intermittent fasting during Ramadan may reduce muscle glycogen stores, impair hydration status, and alter circadian rhythms, leading to declines in strength, endurance, and reaction time [46,49]. However, research also suggests that strategic modifications—such as adjusting training loads, prioritizing nighttime carbohydrate intake, and optimizing hydration—can help athletes sustain high performance levels [13,14]. Additionally, different sports disciplines experience varying levels of performance impact. For example, endurance athletes such as long-distance runners often report greater performance decrements due to prolonged exertion and limited refueling opportunities during fasting hours [22]. In contrast, strength-based

Table 2
Characteristics of original studies and main results related to Ramadan fasting in different sports. ND= Not described.

Author [ref]	Year	Country	Population	Number of participants	Age	Time of data collection	Tests Used for assessment physical performance
Al-nawaiseh et al. [25]	2021	United States	Distance runners	15 males	23.9 ± 3.1 years	Pre-ramadan and the last week of ramadan	Graded exercise test on the treadmill, VO2, heart rate, time to exhaustion, RPE, and running speed
Amjad et al. [23]	2023	Unites states	College student athletes	12 (4 females and 8 males)	19.9 ± 1.4 years	During whole ramadan month	Consensual qualitative research method (open ended interview questions-based method)
Aziz et al. [45]	2017	Singapore	Football players	14 males	21.8 ± 2.4 years	First match the first as familiarization, the second before the ramadan month, the third occasion during ramadan (RAM trial), and the final session was after the ramadan month	Rating of perceived exertion, maximal isometric volumetric contractions of knee extension, maximum contraction of hand grip
Aziz et al. [41]	2019	Singapore	Badminton players	10 males	22.8 ± 3.4 years	Before ramadan and two weeks after starting ramadan	Questionnaire, blood glucose test, lactate test, assessment of perceived exertion (RPE), using Borg's categorical scale of 1 −10
Aziz et al. [49]	2017	Singapore	Soccer players	13 males	20.1 ± 0.9 years	Two matches before the start of ramadan and two matches in last week of ramadan	~80g GPS device and a HR monitor strap
Bencharif et al. [35]	2021	Algeria	Basketball players	32 (gender not defined)	24.9 ± 1.9 years	Three passages during ramadan and one passage after ramadan	Brachial muscle circumference (BMC), body mass index
Bezuglov et al. [43]	2021	Kazakhistan	Elite professional adult soccer players	26 (gender not defined)	Group1 (24.0 ± 2.8) years group 2 (26.0 ± 4.4) years	Before ramadan and third week of ramadan	Instant system for measuring performance
Bougrine et al. [36]	2023	Germany	Handball players	19 females	16.8 ± 0.4 years	Ten days prior to ramadan (R 10), the final ten days of ramadan (R) including both early suhoor R(ES) and late suhoor R(LS) conditions, and the ten days immediately following ramadan (R +10)	Countermovement jumps test (CMJ), modified Agility t-Test (MATT), repeated sprint ability (RSA), and rating of perceived exertion (RPE)
Bouguerra et al. [22]	2017	Tunisia	Highly trained endurance athletes (middle- and long-distance runners)	24 males	28.5 ± 10.6 years	Before, mid and after ramadan	Running time calculated in morning, afternoon and in evening for running
Bouzid et al. [46]	2019	Germany	Soccer players	8 (gender not defined)	21.0 ± 0.4 years	1 week before (BR) and during the fourth week of ramadan (End-R)	Squat jump, countermovement jump, maximal voluntary contraction, and 20 m sprint, and creatine kinase, uric acid, and subjective ratings (feelings scale, quality of sleep, fatigue, muscle soreness and stress) were assessed at baseline and 0, 24, 48, and 72 h following LISTmod.
Brini et al. [39]	2021	Portugal	Basketball players	24 males	Group1 (25.3 ± 2.5) year group 2 (24.8 ± 1.5) year	During whole ramadan but changes accessed during first and fourth week of ramadan	Body composition, sleep habits, and ratings of perceived exertion (RPE) measured
Brini et al. [38]	2023	Tunisia	Basketball players	28 males	Group 1 (26.2 ± 2.1) year group 2 (25.8 ± 2.1) year	First and fourth week of ramadan	Internal load (weekly session rating of perceived exertion (s-RPE), heartrate (HR)), dietary intake, body composition, sleep quality (PSQI survey), well-being indices questionnaire (sleep, fatigue, stress, delayed onset of muscle soreness (DOMS)), and recovery state with the total quality recovery (TQR) questionnaire.

Table 2 (*continued*)

Author [ref]	Year	Country	Population	Number of participants	Age	Time of data collection	Tests Used for assessment physical performance
Carling et al. [48]	2019	United Kingdom	Soccer players	12 (gender not defined)	26.0 ± 4.0 year	Experiment conducted at the beginning of ramadan at different intensity games	Global positioning system device (GPS) sampling at 10 HZ
El-Jaziz et al. [28]	2023	Morocco	Amateur athletes (aerobic sports)	117 (72 females and 45 males)	16.8 ± 1.07 year	Before ramadan, 2 nd week of ramadan, 4th week of ramadan and last days of ramadan	20-M shuttle-run test.
Fashi et al. [16]	2021	Spain	Professional athletes	12 males	23.2 ± 1.3 year	3 weeks after the beginning of ramadan fasting and 2 weeks after the end ramadan fasting.	Muscle function, rating of perceived exertion (RPE), isokinetic knee extension, extension peak torque, flexion, extension average power, flexion average power peak torque, extension total work
Fekih et al. [40]	2020	Italy	Tennis players	27 males	16.9 ± 0.6 year	One week before ramadan and three sessions in 2nd, 3rd, 4th week of ramadan	MAT-agility test
Fekih et al. [42]	2020	Italy	Tennis players	38 males	16.9 ± 0.6 year	Before ramadan, end of first week of ramadan, end of second and 4th week of ramadan	A type of radar gun (sr3800-fp radar vitesse pistolet) was located behind the player, to record the speed of the ball during the execute
Graja et al. [33]	2020	Tunisia	Handball players	12 females	16.5 ± 0.5 year	One week before ramadan, first week of ramadan and last week of ramadan	Body mass, oral temperature (OT), hooper index, vigilance test (VT), epworth sleepiness scale (ESS), five jump test (5-JT), modified agility T-test (MAT) and maximal standing ball-throw velocity test (MSBVT). Running-based Anaerobic sprint test (RAST), rating of perceived exertion (RPE)
Hajji et al. [47]	2023	Yemen	Football players	108 males	25.5 ± 4.4 year	One week before ramadan, during the last week of ramadan, and one week after ramadan	Arabic version of the inventory of coping strategies for competitive sport (ICSCS) scale in each session
Hakoumi et al. [27]	2017	Algeria	Athletes (race of 3000 m)	13 males	21 ± 0.5 to 33 ± 0.5 year	11 days before the period of ramadan, 25th day during the fast period, and 119th day after the fast of ramadan	Chronometric performances related to the manifestation athletes endurance capacity, intermediate distance which permit to note and detect eventual physiological variations. Axillary temperature measurement
Havenetidis [24]	2015	Greece	Running endurance athletes (20m shuttle run test)	20 males	21.7 ± 2.0 year	Periodization training before, during and after ramadan	Training loads from various types of exercises, ratings of perceived exertions (RPE), total quality recovery (TQR) and heart rates
Hsouna et al. [29]	2019	Tunisia	Physically active individuals (fast walking and jogging at least 3days/week)	12 males	21.9 ± 2.4 year	15-days BR, on the first (FR) and last 10-days of ramadan (ER) and 10-days (AR10) and20-days (AR20) after ramadan.	5 jump test evaluation
Khemila et al. [44]	2023	Italy	Football players	11 males	19.27 ± 0.9 year	1 week before ramadan (BR); the second (R2); the third (R3); the fourth (R4) week of ramadan; and 2 weeks after ramadan	Simple (SRT) and choice (CRT) reaction times, mental rotation test (MRT) and selective attention (SA) test were measured before and after wingate test. Rating of perceived exertion (RPE), body composition, dietary intake, profile of mood states (POMS) and Pittsburgh Sleep quality index (PSQI)

(*continued on next page*)

Table 2 (*continued*)

Author [ref]	Year	Country	Population	Number of participants	Age	Time of data collection	Tests Used for assessment physical performance
Lau et al. [30]	2022	Malaysia	Archers	13 males	16.0 ± 1.53 year	One month before ramadan and during Ramadan	The archery shooting performance was evaluated by the total score of 36 arrows shot from a 70 m distance.
Lipert et al. [20]	2021	Lebonan	Endurance athletes	32 males	17 ± 0.5 to 40 ± 0.5 year	Two weeks before ramadan and during ramadan (third week)	Cooper test (covered distance in meters + VO2 max (mlo2/kg/min)), step test (heart rate at rest per minute & heart rate after the step test per minute)
Maneshi et al. [37]	2023	Iran	Volleyball players	49 females	Fasting group (29 ± 7.3 year) non-fasting group (30 ± 5.1 year)	Before ramadan, during ramadan and 2 weeks after ramadan recovering period	Hand strength, muscle power [Sargent's jump and long jump], reaction time, service skill
Mhenni et al. [34]	2021	Tunisia	Handball players	15 females	20.1 ± 1.5 years	One week before ramadan and 2nd and 4th week of ramadan	Hand grip (HG), ball throwing velocity (BTV), modified agility T-test (MAT), and repeated shuttle-sprint and jump ability (RSSJA) tests
Odeh [50]	2023	USA	Swimmers (400 m swam)	8 (4 males and 4 females)	14 ± 0.5 To 16 ± 0.5 year	Ramadan occurred on five consecutive days during the first week of ramadan	Stopwatch, 10 meter measuring tape, dynamometer (100 kg max), iPad, using "reaction time "application, digital sphygmomanometer
Ozbay et al. [26]	2024	Tunisia	Athletes (sprint players)	14 males	22.4 ± 1.8 year	Start of ramadan and day 1, 2nd and 3rd of ramadan	Oxidative, lactic and lactic metabolic pathways were quantified using the PCr-LA-O2 method
Souissi et al. [31]	2013	Tunisia	Judo athletes	11 males	22.5 ± 2.8 year	One week before RIF (BRF), during the second week (SWR) of RIF, and three weeks after RIF	Dynamic balance tests, the movement time, lift-up index, and impact index during the STS test
Yagin et al. [32]	2022	Turkey	Kickboxers	10 (gender not defined)	21.2 ± 1.61 year	Last ten days of ramadan (DR) and 3 weeks after ramadan	Running-based anaerobic sprint test (RAST)
Zarrouk et al. [29]	2016	Tunisia	Karate athletes (black belt 1st dan and regularly engaged in 2 h a day, 5 days a week for at least 3 years)	8 males	17.2 ± 0.5 year	The first study session (S1) was performed one week before ramadan, the second (S2) was performed at the end of the first week of ramadan, and the third (S3) was performed at the end of the last week of ramadan	Simple reaction time (SRT), choice reaction time (CRT), SRTand CRT were evaluated at rest and just after neuromuscular fatiguing exercise

athletes and those engaged in short-duration, high-intensity sports may experience less pronounced effects, particularly if training schedules are adjusted to accommodate fasting constraints [40,42].

Athletes performing RIF have complins and fears about the decrease in level of glucose, muscle protein degradation and decreased glycogen reserves in muscles. However, nearly all studies performed on different categories of sports depict that RIF is not associated with a major adverse effect on physical performance. Lipert et al. investigated the effect of Ramadan fasting on professional male endurance athletes performing running and practicing Ramadan fasting 15 h a day, with environmental temperature of 32°c and 34 % humidity. The physical performance of athletes accessed by cooper test and Harvard step test two-weeks before the start of Ramadan (baseline) and during 3rd week of Ramadan. Physical performance is also associated with the working hours and sleep quality, the subjects with more working hours and less sleep quality showed decreased physical performance during Ramadan (DR) as compared to before Ramadan (BR). Although the athletes with less working hours and good sleep quality showed significant results for cooper and Harvard step test indicating the VO2max and heart rate. Although the overall results indicated a significant reduction in aerobic capacity as well as in cardiorespiratory fitness in athletes during Ramadan [20]. Fashi et al. investigated the effect of Ramadan fasting on the muscle function in athletes. The twelve male athletes who participated in this study were investigated for muscle function three weeks after the start of Ramadan and two weeks after the end of Ramadan. The buffering capacity, muscle function and rating of perceived exertion are determined using the Biodex Isokinetic machine, blood analyzer and Borg scale. During Ramadan fasting athletes' total carbohydrate intake was less as compared to before Ramadan. It seems that a low-carbohydrate substrate during RF impairs muscle performance and reduces the buffering capacity of the blood, leading to fatigue in athletes [16]. Bouguerra et al. conducted a study on elite distance runners (3000 m) for investigating the effect of Ramadan fasting and day timing on the performance of runners. Athletes are divided into three groups with 8 athletes in each group, and each group is assigned an activity time like a morning group, evening group and an afternoon group. The maximal aerobic velocity (MAV), time to exhaustion, VO2 max and performance in 3000-m race measured in each group before the start of Ramadan, during Ramadan and after Ramadan. The results depict that runner who trained during the afternoon exhibited higher MAV and VO2max and greater performance during the 3000-m running test in all sessions as compared to morning and evening group. Moreover, the time required for the 3000-m running exercise significantly increase

between before and mid-Ramadan and significantly decrease between mid- and after Ramadan [22]. Amjad et al. Investigated the effect of Ramadan fasting in Muslim college athletes by using interview methods and questionnaires. The intrinsic and extrinsic challenges identified by using the questionnaire. The Muslim student athletes gave importance to fasting along with their sports activities. The athletic community should seek to better support Muslim student-athletes and respect the importance of fasting during Ramadan. Parties interested in the success of these athletes in sport should improve their understanding regarding Ramadan and the desired support of Muslim student-athletes during fasting [23]. Havenetidis et al. discovered the effects of Ramadan fasting on various training regimes, running endurance performance and recovery in a controlled environment. A total 12 male athletes recruited for this study performed 20-m shuttle run test and parameters evaluated for physical performance being in state of Ramadan fasting. In conclusion, Ramadan fasting does not affect the achievement of daily training goals and the improvement of endurance performance throughout a mixed endurance and strength training programmed where daily undulating periodized models are used allowing athletes to freely select from various recovery modalities above the 26 min span daily, can eventually increase their training recovery under Ramadan fasting conditions [24].

Al-Nawaiseh et al. Investigated the impact of Ramadan fasting on the performance of athlete runners. A total 15 athletes recruited for this study and graded exercise test on the treadmill, VO2, Heart Rate, time to exhaustion, RPE, and running speed were recorded before Ramadan and in the last week of Ramadan. Overall, time to exhaustion and maximal running speed of the distance runners was improved during Ramadan fasting, independent of changes in nutrients intake observed during the current study. Along with proper modulation of training, distance runners' performance can be maintained or even slightly improved following the month of Ramadan fasting [25]. Ozbay et al. discovered that Ramadan fasting has no adverse impact on the sprint players performance when examined their activity during Ramadan period in fasted and non-fasted states. The PCr-LA-O2 method used to access Bioenergetic pathway contributions during sprints and the results depict no differences in performance in fasted and non-fasted states [26].

Hakoumi et al. observed the consequences of Ramadan intermittent fasting in athlete runners and thermoregulation in players. For this study 13 male players involved in 3000-m race and performed 11 days before the period of Ramadan, 25th day during the fast period, and 119th day after the fast of Ramadan. It was concluded that Ramadan intermittent fasting decreased the physical performance of middle-distance race and increase the axillary temperature during the period of fast, what inevitably leads towards a state of dehydration where the sportsman can expose his health to consequences [27]. El-Jaziz et al. Performed a study on athletes involved in athletic sports especially aerobic activities, this study conducted to observe the effects of Ramadan intermittent fasting on player's performance. A total 117 amateur players recruited for this study in which body parameters collected before start of Ramadan and to observe their performance 20-m shuttle run test conducted at 2nd,4th week and last days of Ramadan. Although the tests revealed that Ramadan intermittent fasting decreases the performance capacity in athletes but with good sleep quality index the decrease in performance can be minimized in players [28].

Lau et al. found the differences in performance and mood of archers during Ramadan fasting. The 13 male archers accessed for concentrations using 24 item Brunel mood scale and performance accessed by calculating the total score of 36 arrows, one month before start of Ramadan and during the Ramadan. The results showed that fasting did not affect the shooting performance and mood of the archers. Moreover, fasting helps in reducing the tension of the archers [30].

Fekih et al. performed randomized experimental controlled study for investigating tennis players agility, speed and reaction time during period of Ramadan. A group was allocated motor imagery training during Ramadan to see its impact as compared to control group not being involved in motor imagery training. The results revealed that performance drops in both groups during period of Ramadan but the drop in performance was less for the motor imagery group. Although fasting during Ramadan reduced agility, speed, and reaction time performance for tennis players. A motor imagery-based training program could be an effective approach to reduce the effect of fasting during Ramadan and stabilize physical performance outcomes in terms of agility, speed, and reaction time for male tennis players [40].

Fekih et al. investigated the impact of using motor imagery training in tennis players during period of Ramadan intermittent fasting. The use of motor imagery training instead of physical performance during Ramadan investigated in total 38 players at different intervals of Ramadan month. Although physical performance decreases in Ramadan for tennis players but incorporation of motor imagery training compensate these impacts to a greater extent [42].

Aziz et al. investigated the impact of Ramadan intermittent fasting on the performance in badminton players. The body mass index, food history and other necessary information collected from each player. A 40-min match (2 sets $\times$ 20 min per set in each) in both fasted and non-fasted state match) were conducted during Ramadan period. It was concluded that there are no changes in sleep periods, although there is increased ratio of tiredness and fatigue in fasting players as compared to non-fasting players, but it does not affect the performance capacity in badminton players except the decrease in overhead smash velocity, but it does not decrease the performance level [41].

Odeh et al. investigated the swimmers during Ramadan period to find its effects on the performance of swimmers. The 8 swimmers performing 400-m swimming during Ramadan accessed by using different tests like Stopwatch, 10 Meter-measuring tape, Dynamometer (100 kg max), iPad using “Reaction time “application, Digital sphygmomanometer. As a result on average, participants completed the 400-m swim 3.4 % slower in the fasted Ramadan condition than in the fed condition. So, the RIF negatively impacts the performance of swimmers [50].

The overall effect of RIF on athletes’ performance, health and behavior depicts mixed results as in three studies [23,24,26] the RIF does not impact negatively as the performance grade remains same before, during and after the RIF. Whereas in four studies [20,21,27,28] the performance decreased during RIF and negatively affected the performance and anthropometric measurements. Moreover, in two studies [22,25] the performance parameters increased, this positive trend is associated with the modulation in training timing as performed in afternoon and decreased pre workout along with better sleeping hours. Overall, in tennis the performance decreased but changing the training to motor imagery can bring positive results both in performance and in playing behavior. Moreover, badminton the performance is not affected just by a change in overhead smash quality, although overall performance remains the same.

In individual sports it is observed that there is a reduction in aerobic capacity, decrease cardiorespiratory fitness, increased fatigue and decrement in the physical performance but several studies explain that doing different proper recovery modalities can increase training recovery in RIF in players.

4.2. Ramadan fasting in combat sports

The martial arts represent different types of sports and included in the review are karate, judo, kickboxers and archers (kyudo). Zarrouk et al. Investigated the effect of Ramadan intermittent fasting effect on the neuromuscular performance and muscle fatigue in the young, trained karate athletes. The athletes were involved in control period (one week before Ramadan) to familiarize with the protocol and later during the first and 4th week of Ramadan the athletes were accessed for dietary intake, anthropometric measurements, maximal voluntary isometric contractions (MVC) and a submaximal contraction at 75 % MVC until exhaustion (Tlim) of the right elbow flexors and surface electromyography was recorded from biceps brachii muscle during MVC and Tlim. Simple (SRT) and choice (CRT) reaction times were evaluated at rest and just after the karate game in a random order. The study revealed that Ramadan intermittent fasting does not adversely affect the neuromuscular performance of karate athletes moreover, the RIF does not affect the neuromuscular fatigue and reaction time in elite karate athletes [29]. Souissi et al. discovered the effect of Ramadan intermittent fasting on the postural control performance of judo athletes. In the experimental study 11 male judo players participated and were asked to perform four protocols of dynamic postural control, i.e., sit to stand test (STS), step up and over test (SUO), step quick turn test (SQT), and forward lunge test (FL), one week before RIF (BRF), during the second week (SWR) of RIF, and three weeks after RIF (AR). The results indicated changes in these tests regarding the postural control in SWR group rather than in BRF and AR groups and overall RIF negatively affects the postural control in judo players [31].

Yagin et al. conducted a study on kickboxers to access their performance before and during the month of Ramadan and impact of 30-min nap on performance. To access the performance running-based anaerobic sprint test (RAST) was performed. The results depict that performance lowered during the Ramadan fasting and increases after Ramadan but during Ramadan the performance enhanced due to 30-min nap as compared to participants who did not being involved in 30-min nap [32].

Overall RIF effects differently in different martial arts, as in two studies [31,32] the RIF imposes negatively as decreased performance and postural control in judo and kickboxers, but in the study [29] about karate athletes it does not adversely affect the neuromuscular performance of karate athletes.

4.3. Ramadan fasting in team sports

Graja et al. assessed the effects of Ramadan intermittent fasting (RIF) on cognitive, physical and biochemical responses to exercises in elite young female handball players. A total 12 female athletes recruited for this study; experiment divided into three sessions, one week before Ramadan (BR), during the first week of Ramadan (FWR) and during the last week of Ramadan (LWR). During these sessions, a sequence of tests was performed including Hooper index, vigilance test (VT), Epworth sleepiness scale (ESS), five jump test (5-JT), modified agility T-test (MAT), maximal standing ball-throw velocity test (MSBVT) and Running-based Anaerobic Sprint (RAST) Test. Rating of perceived exertion (RPE) was recorded immediately after the RAST. The main finding of this study was that RF affected negatively subjective sleepiness but not vigilance task. On the other hand, RF affected differently handball specific exercises. The MAT and 5 JT performances were unaffected during RF. Conversely, MSBVT performance increased only in the LWR. However, the decrease of RAST performances during RF was associated with muscle damage and fatigue. Moreover, these alterations seem to be more pronounced during LWR [33]. Mhenni et al. Found the combined effect of Ramadan fasting and day timing on the physical performance of handball players. The 15 elite female team-handball players underwent the hand grip (HG), ball throwing velocity (BTV), modified agility T-test (MAT), and repeated shuttle-sprint and jump ability (RSSJA) tests at 07:00 h and 17:00 h, one week before Ramadan (BR), and during the second (SWR) and fourth week of Ramadan (4WR). The MAT performance improved in the morning during the fasting month of Ramadan. The sleep deprivation in Ramadan may lead to a decrease in team-handball-related short-term maximal physical performance among elite female team-handball players in the evening, inducing a reversal of the diurnal pattern of physical performance [34]. Bencharif et al. worked on finding the impact of Ramadan fasting on basketball players sports activities along with diet and anthropometric measurements. Players got involved in four passages, three passages during Ramadan and one passage after Ramadan. During Ramadan the trainings were carried out before breaking the fast in evening. The players preferred to perform more frequently a period outside Ramadan. The frequency and duration of training decreased during Ramadan to preserve the physical performance of the basketball players and avoid the risk of dehydration during the diurnal period [35]. Bougrine et al. Performed a study on female handball players in which they investigated the effect of Ramadan fasting and timing of Suhoor on the short-term high intensity physical performance capabilities. The study was programmed as to access performance before Ramadan, during the last days of Ramadan and the month after Ramadan, especially during Ramadan early and late Suhoor technique used in association with the physical performance impacts. The Countermovement Jumps Test (CMJ), Modified Agility t-Test (MATT), Repeated Sprint Ability (RSA), and Rating of Perceived Exertion (RPE) were used to determine the performance assessment. The results indicated a decrease in the performing capability in Ramadan intermittent fasting handball players specifically when early Suhoor was introduced in players [36].

Maneshi et al. conducted an experiment on female volleyball players, in which 49 female players were divided into fasting and non-fasting group. The participants got involved in different tests like muscular strength and power agility, reaction time, skills, and perceived exertion tests during four sessions before fasting, two weeks after fasting, and two weeks following recovery. The perceived exertion increased from first day to two weeks and the agility test values decreases after two weeks of Ramadan intermittent fasting. Although the perceived exertion increases in the players during fasting and some physical parameters also changes so Ramadan intermittent fasting shows a negative impact on performance [37].

Brini et al. conducted a study to observe the that increased basketball game frequency in players during month of Ramadan effects the athlete's performance to which extent, for this purpose body measurements, recovery states and sleeping patterns were observed in the basketball players. A total of 28 players were divided into two groups, the one with increased game frequency group and the other one normal game frequency group. The parameters observed during the first and fourth week of Ramadan and when both groups compared for the performance capacities it shows that group performing Ramadan fasting with increased game frequency showed poor performance parameters as compared to the control group [38].

Brini et al. conducted an other study to assess the effects of an additional small-sided games (SSGs) training program during Ramadan Intermitting Fasting (RIF) on technical performance depending on changes in body composition, sleep habits, and ratings of perceived exertion (RPE). A total of 24 participants were divided into two groups with 12 participants in each group. Matches conducted before and during the month of Ramadan and

several tests like technical performance, dietary intake, body composition, sleep quality index (PSQI)survey outcomes, RPE, heart rate (HR), and blood lactate concentration were performed for collecting the results. Our results showed that the technical performance of professional male basketball players was significantly affected at the end of RIF independently of changes in RPE, sleep habits, and body composition. Moreover, the Heart rate significantly decreased during the fourth week of RIF [39].

Overall, the RIF negatively effects the physical performance, anthropometric measurements, fatigue and playing behavior in handball, basketball and volleyball players, although this decrease can be decremented by changing policies like game frequencies, sleeping patterns, playing patterns and dietary intake.

Bezuglov et al. performed a study to explore the effect of Ramadan intermittent fasting on soccer players performances. Two groups of soccer players were taken one consists of 13 Muslim players performing Ramadan intermittent fasting while in other group 13 non-Muslim soccer players were present. Matches conducted before the start of Ramadan and during the third week of Ramadan, running performance of players were reported before and during Ramadan matches and the results shows that conclusion, restrictions in diet and liquid intake during the Holy Month of Ramadan had no negative influence on the running performance of elite Muslim professional adult soccer players during daytime matches [43].

Khemila et al. discovered the effects of Ramadan fasting (RF) on the diurnal variation of physical and cognitive performances at rest and after exercise. In this randomized trial a total 11 players accessed for their performances on five occasions: 1 week before Ramadan (BR); the second (R2); the third (R3); the fourth (R4) week of Ramadan; and 2 weeks after Ramadan (AR) by using different tests like Simple (SRT) and choice (CRT) reaction times, mental rotation test (MRT) and selective attention (SA) test before and after Wingate test. During RF, anaerobic and cognitive performances were unaffected in the morning but were impaired in the afternoon and evening [44]. Aziz et al. showed that Ramadan fasting had adverse effects on the performance of footballers during Ramadan as compared to before and after Ramadan period, despite similarities in dietary intake, sleeping hours and training loads between conditions. The Loughborough Intermittent Shuttle Test (mLIST) was used for the assessment procedure. Moreover, to determine the cause of fatigue different tests like capillary blood glucose, blood lactate, maximal voluntary isometric contractions (MVIC) of the dominant leg and handgrip were measured [45]. Bouzid et al. Observed the effects of Ramadan intermittent fasting on the soccer players for recovery following soccer simulation. Loughborough Intermittent Shuttle Test protocol (LISTmod) performed on players one week before start of Ramadan and on the fourth week of Ramadan. The players performed squat jump, counter movement jump, maximal voluntary contraction, and 20 m sprint test. The performance for these tests decreased for both events before and during Ramadan. So, the results of this study showed that Ramadan fasting did not adversely affect the recovery following soccer match simulation in professional soccer players [46]. Hajji et al. Observed the performance and coping strategies of football players before, during and after Ramadan to access the strategies. It was observed that during Ramadan players adopted a different active coping strategy as compared to before and after Ramadan. Before and after Ramadan players used active coping which is positively associated with the physical performance, while during Ramadan they simultaneously used positive and negative strategies. However the adaptation profile of footballers during Ramadan is unstable and irregular, which is significantly affected by fasting [47]. Carling et al. Investigated the running activity profile in fasted soccer players during a 90-min match. The players commencing play in a fasted state and completing 90mins generally coped physically which might partly be explained by nutritional intake during half-time and/or the adopting of a pacing strategy. The fasting did not negatively affect the performance of soccer players in a running performance as players adopted pacing strategy to save energy in the start and use it at the end of match and it efficiently improved their running performance [48].

Aziz et al. conducted a study to observe the physical activity profile of soccer players observing Ramadan intermittent fasting during a 90-min match. For this study 13 male Muslim soccer players were selected and divided into two groups. Total four matches were conducted, two matches during Ramadan intermittent fasting state and two during non-fasted state. To observe the impact of the total distance covered, perceived exertion tests and questionnaires were used to observe the results. However, Players' physical activity profile during a soccer game was adversely affected by Ramadan fasting and this negative impact was already observed in the initial stages of the match [49].

The overall impact of RIF on soccer players is not adverse, as in four studies the physical performance is not affected negatively, and players adopted different coping strategies to boost performance. Moreover, the games played in daytime shows better performance as compared to the evening group. In football players the RIF imposes negative effects on the performance capabilities.

4.4. Comparison of nighttime eating with ramadan fasting in athletes

Arslan et al. evaluated the eating habits of esports players, although there are differences between esports players and traditional athletes, the study provides useful parallels regarding meal timing, stress-related eating, and performance outcomes. The study found that skipping meals was associated with a higher risk of night eating syndrome, and poor sleep quality was linked to food addiction. This eating behavior had an impact on the performance and the health of players, leading to weight gain and unhealthy habits. As no studies till yet explored the impact of Ramadan fasting in esports players but still as the eating pattern like nighttime eating behavior is somehow similar to Ramadan fasting, it is an association point. The esports players share cognitive and physical stressors with traditional athletes (e.g., performance pressure, prolonged screen time, sleep disturbances), this study supports a broader discussion on the physiological and behavioral effects of altered eating patterns. Esports players have a more sedentary lifestyle than traditional athletes, which may impact metabolism, energy expenditure, and eating habits differently. The findings from this paper explains that Ramadan intermittent fasting might influence meal timing and lead to night eating in athletes [51].

4.5. Religious dietary and fasting practices across different religions

Several dietary practices in different religions like Judaism, Islam, Hinduism, Buddhism, Christianity, and Indigenous religions reflect the multifaceted faces of spirituality. These religious dietary laws have an impact on individuals and communities and implications for well-being and health. Dietary practices based on these religious beliefs have a psychological impact on the mental and physical health of human beings. Sedat & Aydin conducted a study to observe these practices across different religions, it depicts the psychological impacts on well-being. Muslims follow intermittent fasting during the Ramadan period, and Christians also follow fasting during the Lent period, although the fasting time differs between the two religions, it has an impact on health. Now, along with Christians, more Muslims have started participating in sports competitions, which are even held during Ramadan and the Lent

period. Healthcare providers and coaches can better support depending on these dietary practices for health, well-being, and physical endurances [52].

4.6. Impact on physiological and chronobiological changes

Ramadan intermittent fasting leads to several chronobiological changes in the body during fasting. Ramadan fasting did not significantly impact the metabolism of lipids, carbohydrates, and proteins, nor did it alter the average daily levels of hormones in the serum. An increase in serum urea and uric acid was often noted, which can likely be linked to dehydration during this period. Some changes, such as the rise in HDL and apoprotein A1 levels, along with a decrease in LDL, may have positive effects on the cardiovascular system. Nevertheless, research indicated that Ramadan fasting influences the circadian patterns of body temperature, cortisol, melatonin, and blood sugar levels. The intensity of most of these rhythms diminished, and their peak timing shifted. Nighttime sleep quality, alertness during the day, and psychomotor performance suffered a decline. VO2max is an indicator of an individual's cardiorespiratory fitness. Previous research has not found any adverse effects of Ramadan intermittent fasting (RIF) on the aerobic capacity in inactive adults; however, there were observations of decreased heart rate and breathing responses to submaximal exercise during RIF. Although biochemical parameters were not evaluated, numerous studies have noted a rise in the serum levels of protein, urea, uric acid, sodium, and chloride during RIF. These alterations during RIF suggest muscle injury, dehydration, and catabolism among participants, which could be a primary factor contributing to the notable decline in aerobic power during this fasting period. Earlier findings have indicated that the body's metabolism tends to slow down during the day throughout the month of Ramadan [53].

A reduction in venous return and a decrease in catecholamine release have been observed during Ramadan Intermittent Fasting (RIF), resulting in lower sympathetic tone and subsequently leading to declines in blood pressure, heart rate, and cardiac output, which ultimately impact physical and aerobic work capacity during this month of fasting. A diminished glycolytic capacity coupled with a slower recovery of muscle creatine phosphate stores during exercise may also contribute to the reduced aerobic ability observed in fasting participants. Lower carbohydrate intake during RIF could be a factor in the decreased muscle glycogen stores, which in turn affects performance. Agility and flexibility were primarily affected by skill, neuromuscular control, and the coordination of the participant. Research indicated that sleep deprivation occurs during RIF at night, impacting the individual's coordination, alertness, and skill control. Lack of sleep had a direct impact on muscle activity and also influenced physical performance indirectly through changes in mental performance, coordination, and motivation. Therefore, it is possible that the impaired coordination and skill control might not help in compensating for any metabolic decline, resulting in a notable reduction in agility and flexibility scores among the fasting participants in this study [54].

5. Implications for research and practice

Fasting during the month of Ramadan is typically regarded as a crucial religious and spiritual obligation for Muslim sportsmen. Despite the physical and psychological challenges that come with fasting during Ramadan, Muslim athletes strive to overcome these challenges by adopting coping mechanisms that enable them to handle this demanding circumstance. The enhanced fatigue perception and lowering the increased stiffness in the muscles caused by hard training and games played during RIF could be accomplished by increasing the amount of carbohydrates consumed, cutting the length of training sessions, increasing the sleep cycle, or using other recovery techniques. The limitations on meal timing and frequency, along with dehydration during daylight hours, present challenges for Muslim athletes in achieving adequate nutrition and energy levels for their training. Based on the findings from all the studies included in this review, it may be beneficial to adjust the intensity and duration of training to help preserve the quality of workouts during fasting periods. These studies indicate that training could be more effective if it takes place in the morning, soon after the pre-dawn meal. The findings revealed no significant change in Peak VO2, even with a notable decrease in training volume; however, improvements were observed in time-to-exhaustion and running speed when training occurred in a fasting state. Despite the lengthy fasting periods during Ramadan, there were no significant changes in body weight, body fat percentage, or lean body mass. Thus, the combination of shorter daily training sessions and lower intensity, along with decreased caloric expenditure from exercise, may clarify the encouraging outcomes in this study. Coaches should carefully track training outcomes and make necessary adjustments to guarantee their athletes are well-equipped for training and competition most safely and effectively. To address these challenges, many sports organizations and coaching staff have implemented specialized training programs and dietary interventions tailored to Muslim athletes during Ramadan. Future research should focus on longitudinal studies tracking performance adaptations over multiple Ramadan cycles, as well as the role of emerging technologies such as wearable fitness trackers in optimizing training outcomes during fasting periods. To explore this topic further in future studies, it is advisable to track a group of athletes over several Ramadan periods and compare their results from different years to evaluate long-term adaptation trends. Additionally, there is an opportunity to incorporate technology-driven performance training by utilizing wearables (such as smartwatches and heart rate monitors) to assess real-time physiological indicators (including heart rate, sleep quality, hydration levels, and recovery) and to implement mobile applications or AI platforms to record training intensity, fatigue levels, and subjective well-being. Furthermore, dietitians should also design meal plans for athletes based on RIF to enhance physical performance and minimize fatigue.

Author contribution statement

Conceptualization, F.V., T.G., and V.R.S.; methodology, T.G., and F.V.; software, T.G., F.U., and F.V.; validation, T.G., F.U., F.V. and V.R.S.; formal analysis, F.U., F.V. and V.R.S.; investigation, T.G., F.U. and F.V.; resources, T.G., F.U. and F.V.; data curation, T.G., F.U. and F.V.; writing—original draft preparation, T.G., and F.V.; writing—review and editing, T.G., F.U., and F.V.; visualization, T.G., F.U.,F.V. and V.R.S.; supervision, F.V. and V.R.S.; project administration, F.V. and V.R.S. All authors have read and agreed to the published version of the manuscript.

Data availability

PRISMA checklist is available as supplementary file. Other underlying data are available on request to the corresponding author.

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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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Original Article

Regulation of the gut microbiota through meditation and exercise: Potential for enhancing physical well-being across all ages in the twenty-first century

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ABSTRACT

Purpose: This study explores the gut microbiota of Aikido practitioners, a Meditation in Motion discipline, focusing on the integration of moderate-intensity physical activity with meditative practices. The innovative approach examines how these combined activities influence gut health and overall well-being.
Methods: Researchers collected data through anonymous questionnaires including personal information, dietary habits, physical activity levels, and meditation depth, assessed using the Meditation Depth Index. Fecal samples were analyzed to identify correlations between gut microbiota changes and participants biographical and behavioral factors.
Results: The analysis revealed significant alterations in gut microbiota among high-meditation practitioners, with increased levels of *Bacteroides* and *Faecalibacterium* and a slight rise in *Prevotella*. Moderate-intensity Aikido practice was linked to enrichment of Firmicutes. These findings suggest that Aikido promotes a balanced Bacteroidetes/Firmicutes ratio, supporting intestinal eubiosis. The dual focus of Aikido—movement and meditation contribute to improved gut health and enhanced well-being.
Conclusions: Aikido benefits not only physical fitness and muscular function but also cognitive and mental health, particularly stress management, a crucial factor in modern life. While the study has limitations, it provides preliminary insights into the relationship between gut microbiota and Meditation in Motion disciplines. Further research is needed for deep understanding of this topic.

1. Introduction

In the twenty-first century, there has been a profound and accelerated increase in the use of digital technologies across various aspects of society. This transformation has affected nearly every sector, from healthcare to education, business, and entertainment. The development of digital tools and platforms has brought about new ways of communicating, working, learning, and entertaining, creating both opportunities and challenges.[1] In addition, a wide population of both adults and adolescents spend a lot of time on smartphones or computers or watching television, even in their free time. So, considering the advancement of technology, screen time has become a daily activity for all of us.[2] Several studies have demonstrated that a sedentary lifestyle, prolonged screen time, is associated with increased risk for various diseases, such as cardiovascular disease,[3] obesity,[4] and diabetes.[5] Moreover, sedentary behavior also influences sleep patterns and other mental health aspects such as anxiety and depression.[6-8]

According to the World Health Organization (WHO), 28% of the world's adult population (older than 18 years) does not engage in sufficient physical activity (i.e., does not reach the levels of physical activity recommended for good health). Physical inactivity is mainly characterized by women (32% *vs*. 23% of men) and is more pronounced in high-income countries.[9,10]

The importance of physical activity comes from the evidence that it provides people with physical and mental health benefits like improved functional capacity, decreased risks of diseases, improved body composition, weight loss, improved mood, and reduced depression and anxiety.[11–13] In contemporary Western societies, characterized by their high prevalence of stress, cardiovascular and metabolic diseases have become significant public health concerns. This has led to a critical need for effective stress-reducing methods that can be easily utilized by large

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Abbreviations

WHO	World Health Organisation
ISTAT	Istituto Nazionale di Statistica
BDNF	Brain-derived neurotrophic factor
CD	Crohn's disease
IL-10	Interleukin-10
IL-12	Interleukin-12
MEDI	MEditation Depth Index
IPAQ	International Physical Activity Questionnaire
MEDEQ	Meditation Depth Questionnaire
NGS	Next-generation sequencing
PCR	polymerase chain reaction
DNA	Deoxyribonucleic Acid
dsDNA	Double strand deoxyribonucleic Acid
OTUs	Operational taxonomic units
RDP	Ribosomal Database Project
BMI	Body Mass Index
SD	Standard deviation
PCoA	Principal coordinate analysis
ANOVA	Analysis of variance
PA	physical activity
MD	meditation depth
ANOSIM	analysis of similarity
F/B	Firmicutes/Bacteroidetes ratio
OR	odds ratio
CI	confidence interval
SCFA	producing short-chain fatty acids
ENS	Enteric Nervous System
GPCR	G-protein-coupled receptors
HDAC	Histone Deacetylase

numbers of people, readily available, inexpensive, and have minimal side effects. This need was articulated by Henriques et al. in their paper about the anxiety reduction in college students.[14] Physical activity meets all these requirements and additionally, practicing mindfulness meditation or, even better, a sport that involves a meditation part, are considered effective in addressing depression and in preventing diseases.[15]

Regardless of gender or sport type, mindfulness has been found to be positively associated with flow in athletes.[16] Mindfulness-based interventions have been demonstrated to enhance sport-associated physiological activations like salivary cortisol levels, immune responses, and psychological status indicators, as well as sport performances themselves (e.g., shooting and dart throwing performances).[17] Many studies have investigated the role of mindfulness in treating mainly psychological disorders (e.g., anxiety, depression, and stress) but also in relieving pain in chronic medical diseases.[18] From these data, we can conclude that Mindfulness meditation programs show small improvements in anxiety, depression, and pain with moderate evidence, and small improvements in stress and health-related quality of life with low evidence when compared to nonspecific active controls.[19] Recent studies have also correlated a long-term deep meditation practice to an enriched microbiota composition which is in turn related to a reduced risk of psychological issues like depression and anxiety.[20] The world of martial disciplines is rather heterogeneous from different points of view, among which there are the performance pattern, competition, geographical and historical descent, the involvement of the spiritual aspect, and the athleticism level of its practitioners. Studies in literature focus mainly on the best-known martial arts, namely those that involve the competitive aspect. This is the case of the study by Liang et al. (2019), in which the gut microbiota of professional martial arts athletes of different levels, but with similar diets, was analyzed in order to minimize potential confounding effects.[21] Data report that gut microbial diversity and structure of higher-level athletes is significantly higher than that of lower-level athletes. *Parabacteroides* and *Phascolarctobacterium* are the most abundant genera in higher-level athletes and are closely associated with cardiac, metabolic, and mood function.[22–24] In addition, higher level athletes exhibit overexpression of the microbial gene related to carbohydrate metabolism, and this implies greater metabolic capacity of the microbiota. Finally, higher level athletes had significantly higher microbiota capable of histidine metabolism, and this latter amino acid is a key component of muscle carnosine. A recent study by Kang et al. investigated the effects on gut microbiota of two groups of basketball players after 20 weeks of Tai Chi training.[25] Tai Chi can, like Aikido, in fact be counted among the Moving Meditation Disciplines as it is a type of physical and mental exercise that uses breath-controlled body movements. The health benefits of traditional Tai Chi are widely demonstrated in literature, although its role on gut flora appears to be an unexplored field. It is necessary to point out that gut microbiota is influenced by various factors, so data reported from Kang et al. may have been varied by the different exercise approaches. In any case, several data suggest that Tai Chi had a significant effect on the abundance and structure of the gut microbiota: in particular, the species richness of microorganisms was significantly higher in Tai Chi group than that of controls. In addition, the level of Proteobacteria is found to be decreased in the gut flora of the Tai Chi group. The fecal flora of the study group was enriched with butyrate-producing bacteria from the phylum Firmicutes (*Blautia* and *Roseburia*).

In recent years, research has increasingly focused on meditation as a possible influencing factor on gut microbial variability, with systemic effects on the overall health of practitioners. Kanchibhotla et al. investigated the health benefits of the meditation technique “Vaishvanara Agni Meditation”, focusing on the gastrointestinal tract.[26] The data suggested that gastrointestinal health improved significantly by practicing 50 days of meditation. In addition, further improvements in physical strength and psychological mastering were identified. Another study enrolled a cohort of adults who attended a 3-month meditation and yoga retreat.[27] This study assessed the various data like psychometric measures, brain-derived neurotrophic factor (BDNF), cortisol levels, and pro- and anti-inflammatory cytokines before and after the retreat. A decrease in self-reported anxiety and depression was recorded and also an increase in mindfulness. Test results showed an increase in both plasma BDNF levels and the magnitude of cortisol's response upon awakening. Also, levels of anti-inflammatory cytokine IL-10 were increased, while proinflammatory cytokine IL-12 was reduced. In contrast, levels of other proinflammatory cytokines were found to be increased: this could be due to additional influences such as exercise and diet, which varied during the retreat. A study conducted by Sun et al. evaluated the effects on gut microbiota regulation after long term deep meditation (monks).[20] The control group showed greater gut microbial richness, and this could be related to monk's sedentary lifestyle. In any case, several species differ significantly between the two groups: in general, in meditation group the most prominently expressed bacteria were associated with alleviation of mental illness; thus, meditation may play a role in mental health. In addition, the biosynthesis of glycans and lipopolysaccharides increases in the meditation group, which is related to decreased intestinal inflammation, improved barrier function, and reduced infection induced colitis. Several studies have examined the potential benefits of various types of meditations, including mindfulness, in relation to chronic inflammatory conditions such as inflammatory bowel diseases (IBD). The findings of these studies suggest that meditation may contribute to enhancing the quality of life for individuals affected by these conditions.[28] Particularly, several studies

reported a positive correlation between the mindfulness score and health-related quality of life in IBD patients.[28,29] It is important to note that these improvements are also documented in relation to other functional modifications, including levels of C-Reactive Protein and other modulators of inflammatory response.[29,30] Ilan et al.'s study examined the impact of a three-month intervention on Crohn's disease (CD) patients. The combination of cognitive behavioral therapy, mindfulness-based practices, and daily exercise regimens showed promising results. Before the intervention, CD patients showed a microbial profile mostly made up of Proteobacteria (17.71%), Firmicutes (65.56%), Actinobacteria (8.46%), and Bacteroidetes (6.24%). These levels were linked to markers of inflammation and distress. No major changes in diversity were detected after the intervention, but there were significant changes in bacterial abundance among the intervention group. The intervention led to a decrease in bacteria linked to inflammation, and a species of *Lachnospiraceae*, often associated with severe CD, was reduced.[31] Given all these statements and data results about meditation and physical activity on health benefits, the concept of "moving meditation" appears to be a powerful tool in psychophysical well-being. The mind-body approaches combine forms of body movements, typically described as slow and flowing, but may range from a high level of dynamic movement to quiescent static postures, with a focus on breathing and meditation that generate a state of mental and physical relaxation.[32]

The present cross-sectional study aims to evaluate the intestinal microbiota of Italian subjects who practice disciplines characterized by Meditation in Movement (Aikido). Indeed, Aikido may be considered a highly beneficial practice for promoting and maintaining health, due to its inherent sustainability. This discipline is distinguished by the integration of the motor components with the mental and spiritual ones, a process well delineated by meditation, which constitutes an indispensable element of practice. As an activity that can be practiced by anyone, it is also possible to maintain a low motor impact, which makes it suitable and adaptable even for the most vulnerable populations, who can benefit from the positive effects on the body and social relationships. Additionally, Aikido is accessible to all, as it can be practiced in any location, although a mat is the preferred setting. To investigate the impact of Aikido practice on the human intestinal microbiota, an anonymous questionnaire was administered to participants. This questionnaire enquired about personal data, eating habits, levels of physical activity and a literature-validated index of meditation effectiveness, the MEDI (Meditation Depth Index). Subsequently, faecal material was gathered for analysis of microbial composition. Each participant was responsible for collecting faecal samples using dedicated swabs with preservation liquid. The data were analyzed to identify any variations in the intestinal microbiota in relation to personal data, eating habits, levels of physical activity and the degree of depth of meditation. Moreover, the data were compared with data from the literature, which differs from the study population due to the implementation of a strict dietary regimen, participation in competitive sports, and the absence of meditation.

2. Material and methods

2.1. Ethical approval

The study protocol was approved by the Institutional Review Board of the University of Rome "Foro Italico" (approval number 204/2024). Aikido practitioners were invited to participate after their scheduled training sessions. During the recruitment phase, the researchers clearly explained the study's objectives, procedures, and ethical safeguards, including data anonymity and confidentiality. All participants were required to provide written informed consent prior to inclusion in the study, in accordance with the Declaration of Helsinki.

2.2. Study design

Each participant was provided with a faecal swab (Copan Italia S.P.A., Brescia, Italy), accompanied by instructions for the collection of a faecal sample. Participants were requested to deliver the swab on a scheduled day within 2 h of collection. Subsequently, the samples were stored at 4–8 °C in a refrigerated container and transported within 24 h to the laboratory of the University of Rome 'Foro Italico', where they were processed using a previously validated protocol for DNA extraction from faecal traces. Additionally, a salivary swab was delivered and stored by the procedure above. The study targeted adult Aikido practitioners attending regular classes. A total of 28 individuals were approached and provided with standardized sampling kits and detailed instructions. Of these, 25 consented to participate and completed all the required procedures, including informed consent, questionnaire submission, and biological sample collection. Two samples were excluded from the final analysis due to protocol non-compliance (e.g., improper labeling or delayed sample delivery). Therefore, the final dataset consisted of 23 fully eligible and analyzable participants. Inclusion criteria were: regular Aikido practice, consent to provide samples and complete the survey, and absence of antibiotic/probiotic use or gastrointestinal procedures in the previous three months. All data were anonymized using a code derived from non-identifiable personal information and matched across sample and questionnaire entries (Fig. 1).

2.3. Questionnaire

The participants were requested to complete a questionnaire, administered via the EUsurvey platform, which requested information regarding their age, sex, weight, height and any specific dietary regimes they may adhere to (e.g. vegetarian or vegan). It was made clear that providing this information would not lead to the exclusion of any participants. Additionally, intercurrent chronic diseases, pregnancy, food intolerances, concomitant infections (and the associated use of antibiotics and probiotics) or gastrointestinal surgical procedures in the previous three months were investigated and represented exclusion criteria. The 9-item questionnaire developed by Martínez-González et al. and the short version of the International Physical Activity Questionnaire (IPAQ) were employed to assess participants' dietary habits and usual physical activity levels.[33,34] Furthermore, a 35-item questionnaire, designated the Meditation Depth Questionnaire (MEDEQ), was administered for the purpose of evaluating the depth of meditation practiced by each participant.[35] The initial questions of the questionnaire pertain to the methodology and duration of meditation, encompassing both the number of years spent practising and the minutes dedicated to each session. The second part of the questionnaire comprises 30 questions designed to elicit information about the physical and mental sensations experienced by the subject during the meditation session. This data is then used to calculate the MEDI, a quantitative measure of the depth of meditation.[35] The MEDI and MEDEQ are two instruments developed by Piron (2008).[36] For each question, respondents were required to indicate their level of agreement using a scale ranging from 0 (indicating a lack of agreement) to 4 (indicating a high level of agreement). The total score was obtained by summing the factors that were perceived to be conducive to meditation.[3,4,6–9,11,12,15,16] The total score is obtained by summing the scores for factors 16, 17, 19–30, and items 1, 4, 9, 12, 13, and 18, which are inversely related to meditation. It should be noted that the total score is not related to the time factor, but rather to the physical and mental sensations perceived during meditation.[35]

2.4. Collection data

The sample size ($n = 25$) provided 84% power to detect a difference with an effect size of 0.7 at alpha 0.05 using paired two-tailed *t*-tests.[37]

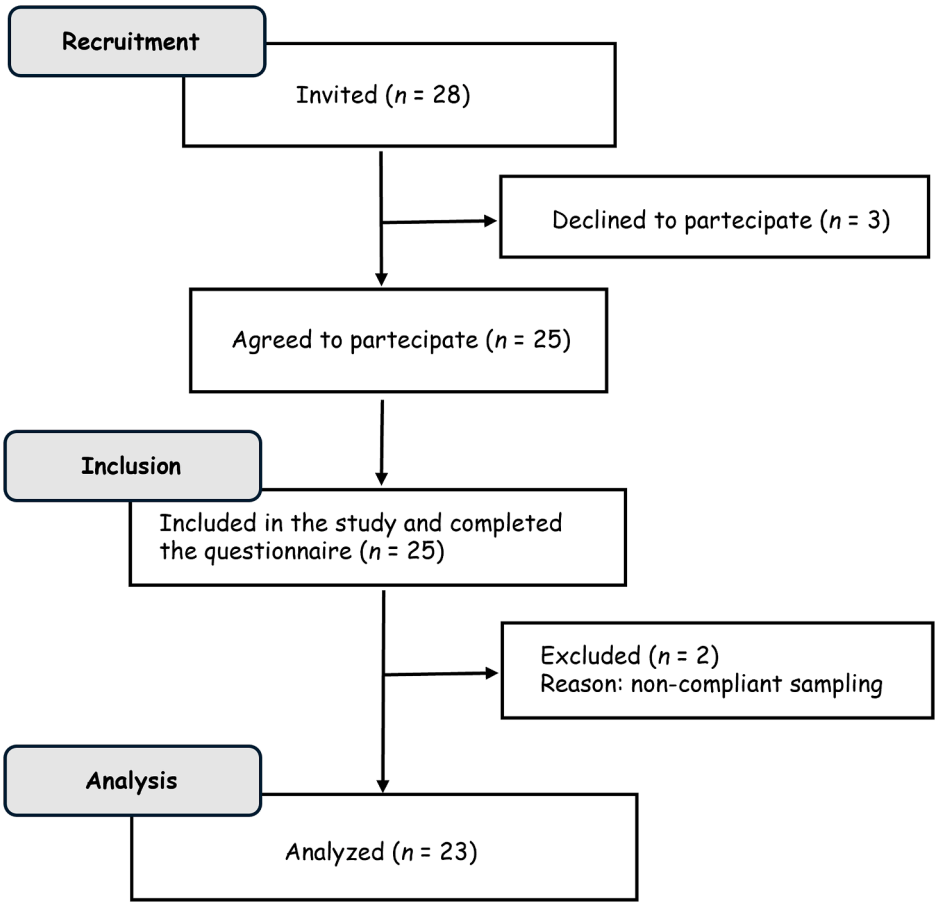


Fig. 1. Flowchart of the study. Recruitment, inclusion, and analysis phases of participants enrolled in the Aikido and meditation-based microbiota study. Of 28 individuals invited, 25 agreed to participate. Two were excluded due to non-compliant sampling, resulting in 23 participants in the final analysis.

Data was collected following an invitation proposed at the end of class. Those who accepted the invitation were provided with the material on pre-determined days. The material consisted of: information letter, informed consent form, instructions, test tube for faecal sampling, test tube for saliva sampling. Labels were placed on the test tubes and linked to complete the questionnaire. The faecal and salivary material for analysis was collected independently, following detailed instructions on the collection method, and kept refrigerated until delivery on pre-established days and times. To ensure anonymity, each participant was identified by a code consisting of the first three letters of the mother's name and the father's birth year (e.g. FRA1967), which was unknown to researchers. The identification code was placed on each tube using the provided labels and inserted into the questionnaire, which allowed the cross-analysis of data. The questionnaire, which included both open and closed questions, was administered via a link on the EUSurvey platform, the official European Commission tool for managing online surveys.

2.5. Data analysis

A faecal swab (Copan Italia S.P.A., Brescia, Italy) was provided to each participant, along with written instructions for stool sample collection. Participants were instructed to deliver the sample on a pre-determined date, within 2 h from collection. The samples were temporarily stored at 4–8 °C in a refrigerated container and transported within 24 h to the laboratory at the University of Rome "Foro Italico". Upon arrival, faecal samples were weighed, and DNA was extracted using a previously validated protocol for microbial DNA isolation from faecal traces. DNA was then purified and normalized prior to sequencing. Amplicon libraries targeting the V1–V3 region of the 16S rRNA gene were prepared following the *16S Metagenomic Sequencing Library Preparation Guide* (part# 15044223 Rev. A; Illumina, San Diego, CA, USA).[38,39] Tagged polymerase chain reaction (PCR) products were generated by two-step PCR using unique barcoded primer pairs. In this strategy, the adapter-containing target primers were used in the first PCR reaction to amplify the target gene (16S rDNA), and this product was then used in the second PCR using the barcode-containing primers to identify the individual sample. Each amplification reaction had a total volume of 25 μL, containing 12.5 μL of KAPA HiFi Hot Start Ready Mix (Roche, Pleasanton, CA, USA), 5 μL of each primer (1 μM) and 2 μL of template deoxyribonucleic Acid (DNA). Reactions were performed on a Techne® TC-PLUS thermal cycler (VWR International, LLC, Radnor, PA, USA). After amplification, 5 μL of PCR product from each reaction was used for agarose gel electrophoresis (1%) to confirm amplification. The final concentration of clean DNA amplicon was determined using the Qubit PicoGreen dsDNA BR Assay Kit (Invitrogen, Grand Island, NY, USA). Libraries were prepared using the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) and the iSeq100 sequencing platform (Illumina, San Diego, CA, USA). Raw sequencing data were processed using a customized bioinformatic pipeline based on the Galaxy platform, integrating quality control and taxonomic assignment tools. Initial quality assessment of FASTQ files was performed using FASTA/Q Information Tools and Mothur. Reads were filtered to exclude low-quality sequences (length $<$ 200 nt), ambiguous base calls, and chimeric sequences. High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity, using a de novo clustering approach. Representative sequences from each OTU were taxonomically classified using the Ribosomal Database Project (RDP)

Classifier (version 2.5), which assigns taxonomic ranks based on a naïve Bayesian algorithm. OTUs defined at 97% similarity were considered species-level units, while 95% similarity was used for genus-level classifications.

Additionally, sequencing reads were analyzed in parallel using the Illumina BaseSpace 16S Metagenomics App (version 1.0.1), which applies an alternative taxonomic classification pipeline based on the curated May 2013 release of the Greengenes reference database. Only taxa with a relative abundance above 0.1% were included in downstream statistical analyses.

2.6. Statistical analysis

A descriptive analysis was conducted on the information collected. Weight and height values were used to calculate the BMI (Body Mass Index). BMI, an anthropometric indicator, is calculated as the ratio between body weight (expressed in kilograms) and the square of height (expressed in meters). Other parameters that define the cohort are age, type of diet and levels of habitual physical activity and expressed as mean ± standard deviation (*SD*) and ranges. Participants were also grouped according to their meditation score with 3 score levels: 11 with a low score (0–60 – MED 0) (44%), 7 with a medium score (60–80 – MED 1) (28%), 7 with a high score (80–120 – MED 2) (28%) and level of Aikido practice. The meditation score is related to the physical and mental sensations perceived during practice, rather than the time factor. Relative abundances of community members were determined with sparse data and summarized at each taxonomic level. The proportion of the gut microbiome at each taxonomic rank, such as phylum, order, class, family, and genus, was determined using the RDP classifier and the Greengenes database. Briefly, the relative abundance of the bacterial community was determined across 29 phylotypes (%abundance = [number of sequences per phylum or genus/total sequences per sample] × 100). Subsequent mathematical analyses excluded the 24 rarest phyla (i.e. those with a relative abundance lower than 0.1% across all samples). Alpha and beta diversity were 56 calculated using Primer7 software at a sequence similarity level of 97%. For alpha diversity, the Shannon index and species-level evenness index were calculated. Principal coordinate analysis (PCoA) was performed using the Primer7 software platform to investigate dissimilarity between the groups.[40–42] Student's *t*-test was used to compare bacterial diversity and relative abundance of major bacterial phyla and genus (representing 90% of the fecal bacterial component) of samples from participants with different Aikido levels and meditation levels. Analysis of variance (ANOVA) with a Bonferroni post hoc test was used to assess differences between participants grouped according to habitual physical activity (PA) levels and to compare lifestyle subgroups obtained by combining meditation depth (MD) adherence and PA. The statistical significance of factors potentially contributing to compositional differences between microbiota samples were also tested with PRIMER software (version 7) using the nonparametric permutation analysis of similarity (ANOSIM) function and the default setting. To identify possible determinants of microbiome composition, two linear regression analyses were performed with age, gender, BMI, diet, and Aikido level as independent variables and Shannon index or Firmicutes/Bacteroidetes ratio (F/B) as outcomes. A stepwise procedure was used to adjust for possible confounders. A p-value < 0.05 was considered statistically significant. Data were analyzed with IBM SPSS version 25 for Windows (SPSS, Chicago, IL, USA).

3. Results

Cohorts are particularly homogeneous, and consistent with these results, also *t*-test produced no significant dissimilarity for BMI, sex, age, level of education, smoking, alcohol, diet, and Aikido level ($R = -0.011$, $p = 0.5$; Table 1).

A total of 1 688 571 sequences were produced by sequencing 23 samples (one sample was not in line with prerequisites required by the analysis). The number of sequences for each sample ranged from 20 000 to 92 000, leading to the identification of 471 OTUs defined at 99% identity. Regression analyses showed that only meditation score was inversely proportional to the Shannon index ($p = 0.02$, odds ratio (*OR*) $= -0.08$, 95% confidence interval (*CI*) $= [-0.16$ to $-0.01]$), as shown in Fig. 2 a where on the x-axis 0 = low levels of meditation, 1 = medium levels of meditation, and 2 = high levels of meditation. Data analysis showed that alpha diversity of practitioner's gut microbiota was similar according to the Aikido level (Fig. 2b).

Alpha diversity indices Chao1 ($p = 0.796$) and ACE ($p = 0.886$) were not significantly different when compared between group 1 (high level of Aikido) and group 2 (insufficient level of Aikido). However, the alpha diversity of gut microbiota was lower in higher levels of meditation (Fig. 2). From data analysis, training intensity and significant differences in biodiversity richness based on Chao1 ($p = 0.006$) and ACE ($p = 0.004$)

Table 1
Socio-demographic characteristics of the sample ($n = 24$).

Variable	Value	Percentage
Gender		
female	9	37.5
male	15	62.5
Age		
18–30	1	4.2
31–65	20	83.3
> 65	3	12.5
Educational level		
Middle school graduation	1	4.2
High school degree	9	37.5
Bachelor's degree	1	4.2
Master's degree	9	37.5
PhD	4	16.7
Smoking		
No	16	66.7
Yes	8	33.3
Smoking frequency		
At least once/day	6	75
4-6 times/week	1	12.5
Maximum 3 times/week	1	12.5
BMI		
Normal weight	17	70.8
Overweight	6	25
Obese	1	4.2
Diet		
No diet	10	41.7
Mediterranean	8	33.3
Vegetarian	3	12.5
Requested by physician	1	4.2
Caloric restriction	1	4.2
Other	1	4.2
Alcohol consumption		
Often (> 5 times/month)	11	45.8
Sometimes (3–4 times/month)	10	41.7
Occasionally (1–2 times/month)	3	12.5
Aikido practice		
< 300 min/week	14	58.3
> 300 min/week	10	41.7
General physical activity		
Partially active	4	16.7
Physically active	20	83.3
MEDI score		
Low (0–60)	10	41.7
Medium (60–80)	7	29.2
High (80–120)	7	29.2
Meditation time		
< 60 min/week	13	54.2
> 60 min/week	11	45.8

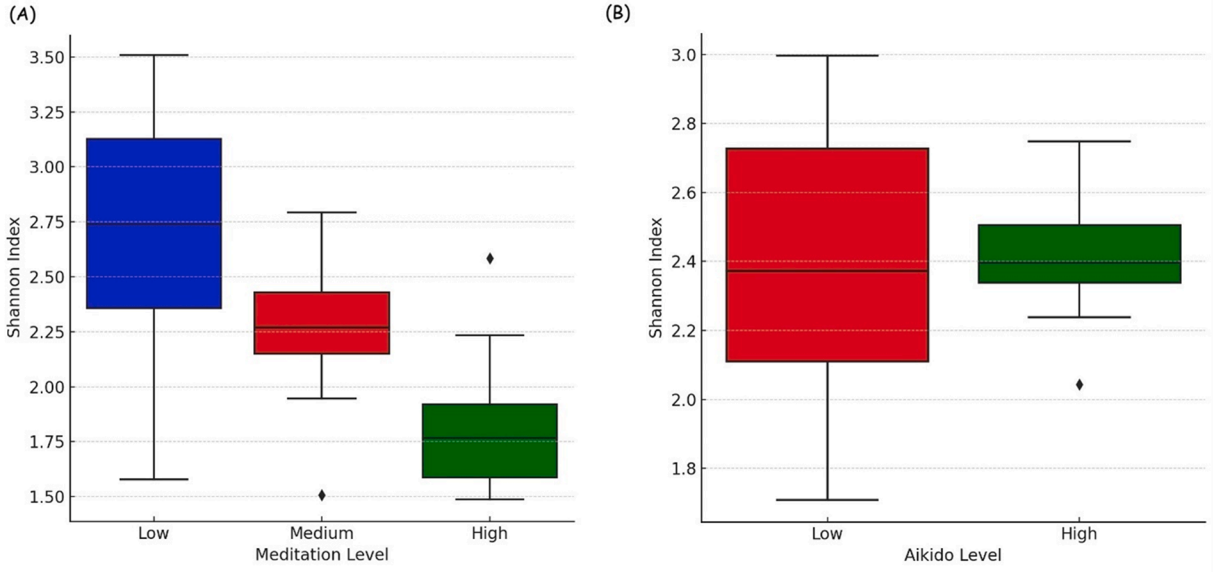


Fig. 2. Shannon Index Distribution by Meditation and Aikido Practice Levels. Box plot showing the distribution of the Shannon index in relationship between Aikido levels and Meditation depth. The box border closest to zero indicates the 25th percentile, the line inside the box represents the median, and the box border farthest from zero indicates the 75th percentile. The whiskers above and below the box indicate the 10th and 90th percentiles. A) Shannon value trend in three levels of Meditation. A significant reduction in alpha diversity is observed in the high meditation group, suggesting a potential compositional shift in gut microbiota associated with deeper meditative practices. B) Shannon value trend in two levels of Aikido. The Shannon index appears slightly higher in the low Aikido practice group compared to the high practice group, although the medians are similar (~2.4). The "Low" group shows greater variability (wider range and presence of outliers), while the "High" group appears more homogeneous. However, the difference is not statistically significant, suggesting that the level of Aikido practice may not substantially influence gut microbiota alpha diversity.

indices. Beta diversity was demonstrated as PCoA based on Bray Curtis algorithm by clustering for different levels of meditation (Fig. 3). Statistically significant differences were found in gut microbiota composition based on Aikido and Meditation levels ($p = 0.001$). Differences were recorded for meditation level ($p < 0.1$). Principal Coordinates Analysis (PCoA) based on Bray–Curtis dissimilarity revealed distinct clustering patterns among the three meditation groups, with partial overlap between groups 0 and 1, while group 2 appeared more dispersed along the first principal coordinate (Fig. 3). The variable representing Aikido practice (AIKIDO) did not lead to a complete separation of the samples, although some directional shifts were noticeable, particularly within meditation group 1. This pattern suggests a potential interaction between physical-meditative activity and gut microbiota structure, although not strong enough to drive a dominant separation. These observations are further supported by ANOSIM analysis for the Meditation score, which showed a moderate but significant effect ($R = 0.350$, $p = 0.001$).

The first two PCoA axes explained approximately 33.62% and 19.4% of the total variance in microbial composition.

The biplot includes vectors representing the top 10 most variable genera, indicating their correlations with the PCoA axes. For example, *Roseburia* and *Blautia* vectors point along the PCoA1 axis, suggesting a higher abundance of these genera in samples with positive PCoA1 scores—possibly associated with MED groups 0 or 1. In contrast, *Anaerostipes* and *Lachnospira* align more strongly with PCoA2, contributing to variation particularly within group 2. These directional trends highlight that specific genera may act as key microbial drivers of group-level differences, and potentially serve as biomarkers in future interventional studies.

With regard to Aikido practice, subtle compositional differences were also observed and supported by ANOSIM analysis ($R = 0.250$, $p = 0.001$). At the phylum level, the most abundant taxa across all groups were Firmicutes (53%) and Bacteroidetes (34%), followed by subdominant phyla including Proteobacteria (6.7%), Actinobacteria (3.2%), and Verrucomicrobia (2.0%), together accounting for over 98% of the gut microbiome (Fig. 5).

When microbial composition was stratified by meditation level, phylum-level distributions showed notable trends (Fig. 4). In group 0, the predominant phylum was Firmicutes (63%)—now classified under the revised nomenclature as *Bacillota*—followed by Bacteroidetes (25%) (*Bacteroidota*), Actinobacteria (5%), and Proteobacteria (6%). In group 1, the abundance of Firmicutes decreased to 50%, with Bacteroidetes increasing to 42%. This trend continued in group 2, with Firmicutes at 48% and Bacteroidetes rising to 45%. Actinobacteria and Proteobacteria showed a corresponding decrease across groups, further confirming a shift in microbial dominance profiles with increasing meditation engagement. At the taxonomic genus level, 103 taxa were present with a relative abundance greater than 0.1%, where *Bacteroides* (18.4%), *Alistipes* (7.0%), *Faecalibacterium* (4.5%), *Blautia* (2.4%), *Ruminococcaceae* UCG-002 (2.4%) and *Roseburia* (2.2%) were the most abundant bacteria in the intestine. *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus* and *Collinsella* represented the top five genera (whose proportions were greater than 5%). Heatmap, in Fig. 5, shows the relative abundance (%) of the top 20 most represented bacterial genera across three meditation levels (Low, Medium, and High). The most dominant genus overall was *Bacteroides*, with relative abundances ranging from 26.98% (Medium) to 34.39% (High), followed by *Faecalibacterium*, *Prevotella*, *Ruminococcus*, and *Collinsella*. Notable shifts in microbial composition were observed across meditation levels: for example, *Prevotella* peaked at Medium meditation (17.01%), while *Faecalibacterium* and *Bacteroides* showed a progressive increase from Low to High meditation. Other genera such as *Sutterella*, *Collinsella*, and *Ruminococcus* showed higher relative abundance in the Medium group, whereas genera like *Streptococcus*, *Lachnospira*, and *Alistipes* remained relatively stable across all levels. Fig. 5 illustrates how specific microbial genera may fluctuate in relation to the depth of meditative engagement, suggesting a potential link between meditation practice and gut microbiota composition.

A comparison of the most abundant bacterial genera (relative abundance > 1%) between the *Aikido* and *Meditation* groups revealed both overlapping and distinct microbial patterns (Fig. 6). In total, 10

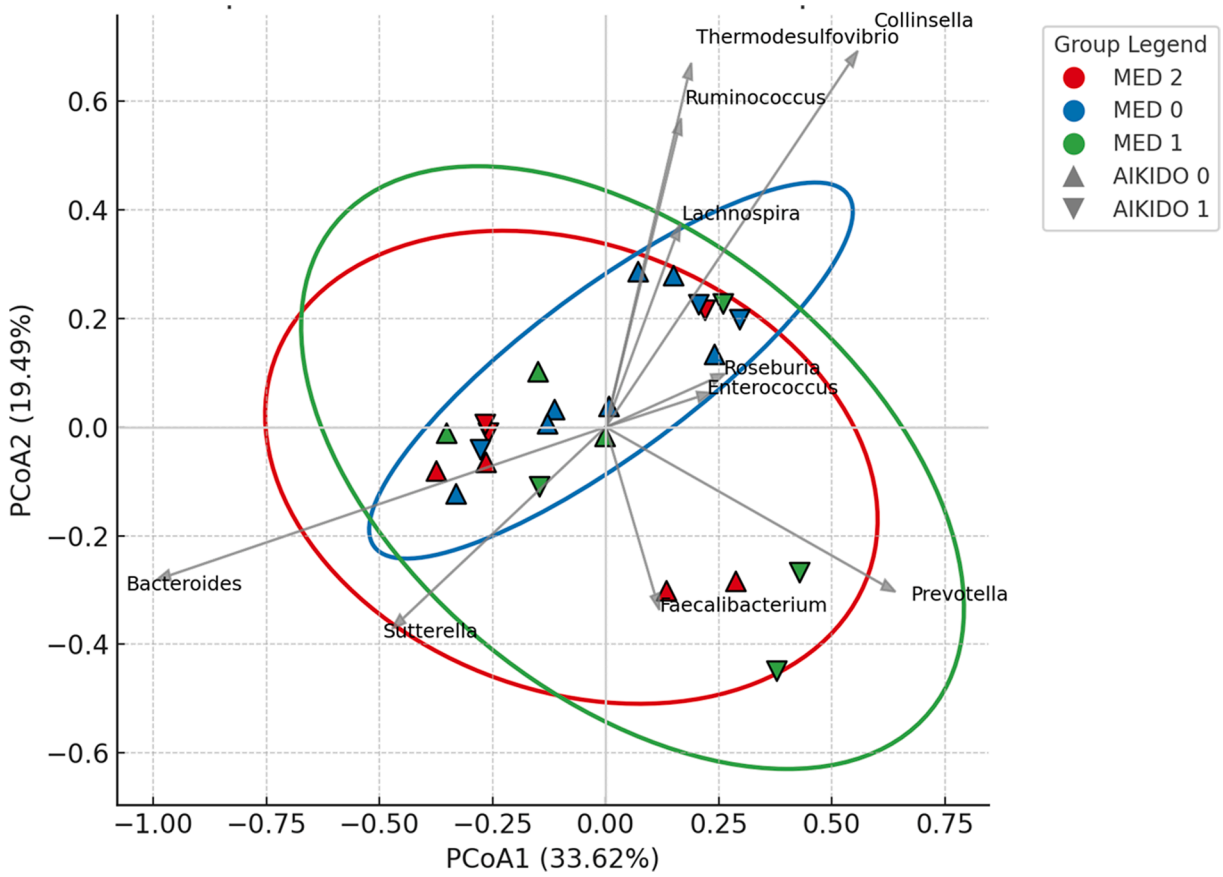


Fig. 3. Principal Coordinates Analysis of Gut Microbiota by Meditation and Aikido Practice. Principal Coordinates Analysis (PCoA) biplot based on Bray–Curtis dissimilarity of genus-level microbial profiles. Samples are color-coded by MED groups—red for group 0, green for group 1, and blue for group 2—and shaped by AIKIDO practice status (▲ for non-practitioners, ▼ for practitioners). Ellipses represent 95% confidence intervals for each MED group, delineating the dispersion of samples within each group. Arrows indicate the Spearman correlation vectors of the 10 most variable genera, illustrating their directional associations with the first two principal coordinates. PCoA1 and PCoA2 explain XX% and YY% of the total variance, respectively.

genera exceeded the 1% threshold in the Aikido group, while 13 genera did so in the Meditation group. Notably, seven genera were found in both groups: *Prevotella*, *Bacteroides*, *Roseburia*, *Enterococcus*, *Ruminococcus*, *Faecalibacterium*, and *Alistipes*, all of which are common and relevant members of the human gut microbiota. By analyzing the various distributions and fluctuations of the percentages of these genera in various levels of meditation depth and Aikido frequency, we observed that some of these genera tend to change within Meditation group. Also, these changes are statistically significant ($p = 0.000\ 1$), while lower fluctuations are recorded for Aikido practice levels. In contrast, three genera were exclusive to the Aikido group (*Phascolarctobacterium*, *Oscillibacter*, *Dialister*), and six were unique to the Meditation group (*Collinsella*, *Sutterella*, *Lachnospira*, *Parabacteroides*, *Butyrivibrio*, *Lachnoclostridium*). These differences suggest that distinct microbial profiles may be associated with specific lifestyle or practice-related factors, such as physical movement, postural stability, and meditative engagement. *Prevotella* was found slightly increased compared to the group with the lowest meditation score, and these results are also shown in Fig. 6.

4. Discussion

Human gut microbiota comprehends a large community of commensal, symbiotic and pathogenic microorganisms like bacteria, fungi, virus and protozoa. This serves a lot of important functions like physiologic ones, immune function, nutrition and metabolism of the host and thus. Its composition is crucial in keeping a state of health. It is therefore considered an influential “hidden organ”. Indeed, numerous studies have shown the correlation between a microbiota alteration and a range of disorders such as obesity, inflammatory bowel diseases, autism, cardiovascular disease, osteoporosis and cancer.[43–47] The human gut microbiota's composition is influenced by numerous factors such as environment, diet, lifestyle, health and disease status, and drugs.[48] Recent studies have shown that exercise is also an important modulating factor.[49,50] Studies concerning the effects of physical activity on gut microbiota are an entirely new field, and while mechanisms of influence are not yet entirely clear. Preliminary studies have revealed a bidirectional relationship that has to be further explored. These first studies were conducted on animal models and reported beneficial changes in microbiota composition of trained rats compared with controls.[51] In addition, a protective function of exercise against negative consequences of high-fat diets has been identified.[52] In addition to animal experiments, studies on professional athletes are also increasing to investigate the unique characteristics, benefits and diversity of their gut microbiota.[53] Indeed, regular physical activity should be considered as a valid method to maintain microbiota eubiosis (or rebalance any dysbiosis), thus improving health status. Further and more in-depth studies on the specific changes produced by physical activity on the composition of gut microbiota could be useful to explore new approaches for the treatment of metabolic and inflammatory diseases, in which the microbiota plays a key role. In this regard, data reported in literature show that moderate-intensity physical activity can provide greater benefits to gut microbiota than that given by high intensity or not at all exercise. In fact, physical activity leads to increased abundance of genera producing short-chain fatty acids (SCFA) and lactic acid-producing bacteria and, concomitantly, improves gut microbial diversity resulting in increased *Akkermansia muciniphila* and *Oscillospira*.

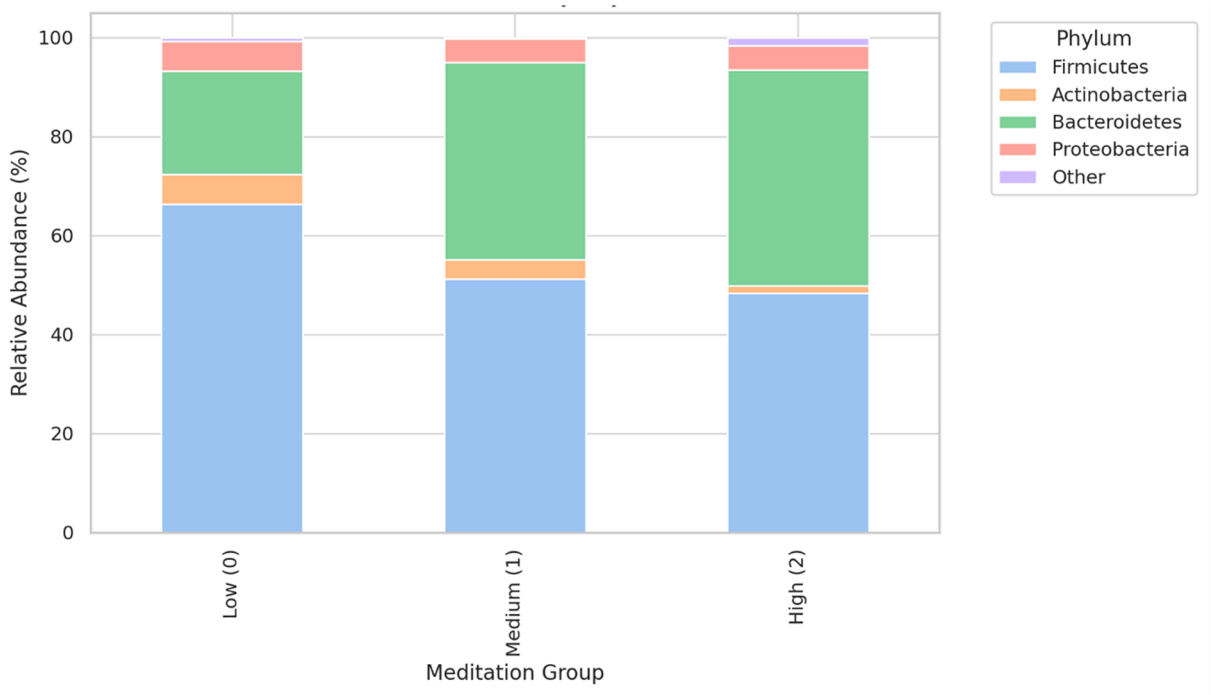


Fig. 4. Normalized relative abundance of bacterial phyla across meditation levels. Stacked bar plot showing the average composition of bacterial phyla in participants grouped by meditation depth: low (0), medium (1), and high (2). Only phyla with a mean relative abundance $\geq$ 1% across all samples are displayed individually; remaining taxa are grouped under "Other".

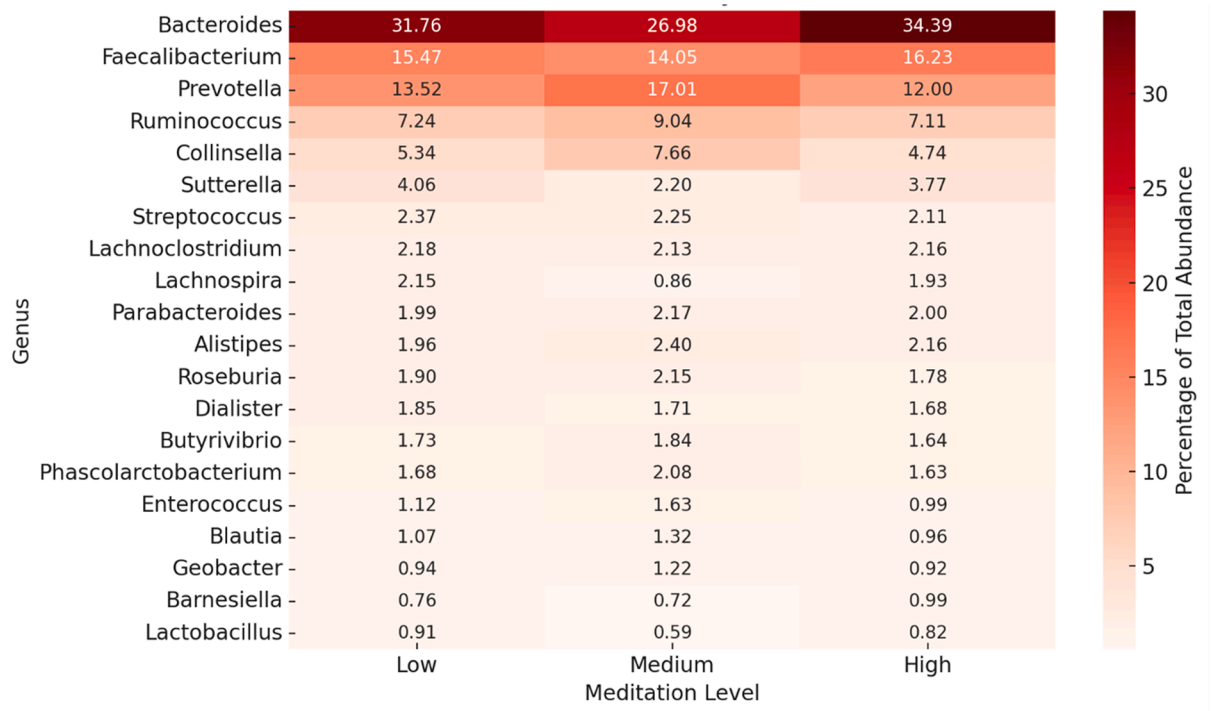


Fig. 5. Relative contribution of bacterial genera to total community abundance across meditation levels. This heatmap shows the percentage contribution of selected bacterial genera to the total abundance of gut microbiota in participants stratified by meditation level (Low, Medium, High). Values represent the proportion of each genus within the total bacterial load of the corresponding group, not normalized by row. Notably, *Bacteroides* consistently dominates the microbial composition, while genera such as *Faecalibacterium*, *Prevotella*, and *Collinsella* show variations across meditation levels, suggesting potential microbiota shifts linked to meditative practice intensity.

In contrast, intense exercise appears to have a negative effect on the gut microbiota.[54] This is an important finding for the present work, as it lends even more relevance to the use of Aikido as a possible tool for a positive influence on gut microbiota, with systemic effects on overall health. Indeed, Aikido is a discipline suitable for everyone, offering an extremely flexible performance that can be adapted to moderate

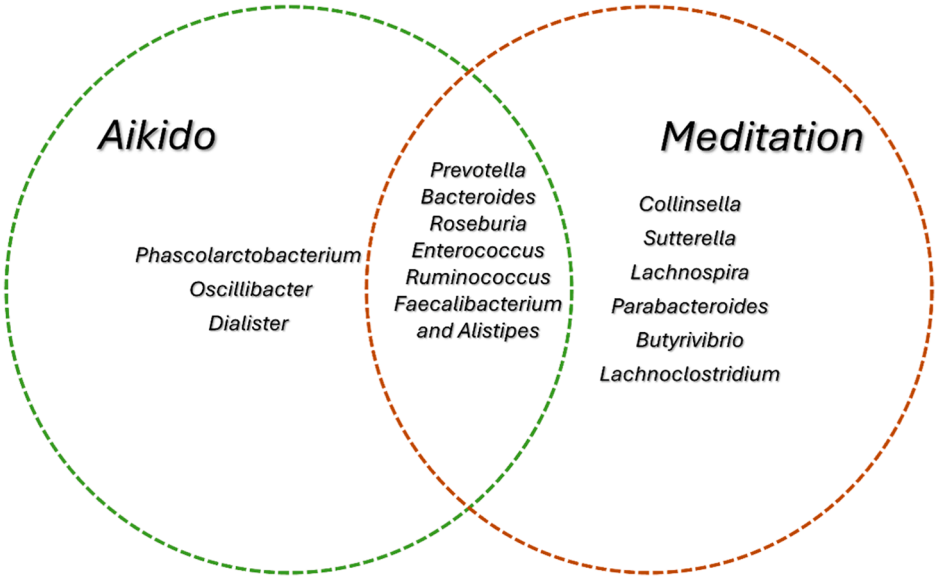


Fig. 6. Shared and Unique Bacterial Genera in Aikido and Meditation Groups. Venn diagram showing the distribution of bacterial genera (cutoff > 1% relative abundance) between the Aikido and Meditation groups. Seven genera—*Prevotella*, *Bacteroides*, *Roseburia*, *Enterococcus*, *Ruminococcus*, *Faecalibacterium*, and *Alistipes*—were shared between the two groups. The Aikido group uniquely exhibited *Phascolarctobacterium*, *Oscillibacter*, and *Dialister*, while the Meditation group was characterized by *Collinsella*, *Sutterella*, *Lachnospira*, *Parabacteroides*, *Butyrivibrio*, and *Lachnoclostridium*. These patterns may reflect the influence of physical and/or meditative practices on the gut microbiota composition.

intensity regimen. Furthermore, meditation practices, ranging from mindfulness to deep breathing exercises, induce a state of relaxation and focused attention. While forms of meditation have been present in many Eastern cultures for millennia, these practices are recently gaining increasing interest also in Western cultures. In addition to the spiritual benefits, meditation practice leads to physiological changes such as improved brain function, immune response and inflammatory processes. Contextually we may appreciate a positive effect on the control of diabetes, fibromyalgia, hypercholesterolemia, psychological disorders and a reduction in heart rate and blood pressure which promotes stress reduction and general well-being.[55] Stress is a well-known aggravator of gut dysfunction, affecting gut motility, barrier function, and microbiota. Meditation, through its stress-reducing effects, can positively influence gut health; in particular, it helps restore the balance of gut bacteria, improves gut barrier function, and reduces inflammation.[56] Thus, the combination of mindfulness with daily exercise can significantly impact the psychological symptoms and inflammation through microbial changes in chronic pathologies like inflammatory bowel diseases, diabetes, cancer, psoriasis, etc.[31,57,58] New research underscores the positive impact of regular meditation on gut health. Specifically, one study evaluated effects of years of deep meditation practiced by Tibetan monks on gut microbial regulation. In this study, the phyla Bacteroidetes and Firmicutes (currently referred to as Bacteroidota and Bacillota) were dominant in both groups, as would be expected. However, Bacteroidetes were significantly enriched in high level of depth meditation (29% *vs.* 4%), which also contained abundant *Prevotella* (42% *vs.* 6%) and a high number of *Megamonas* and *Faecalibacterium*. It seemed that the bacteria present in subjects performing meditative exercises would be associated with genera implicated in recovery from psychological or neurological disorders.[59] Based on this scientific evidence, in this work we focused on the assessment of gut microbiota in a sample of subjects practicing Meditation in Motion (Aikido) disciplines. The interest in this discipline is related to its conciliation of both motor and spiritual components, which make it a widely used practice in population of all ages. Being an activity "for everyone" means the possibility of practicing it in a low impact mode, making it suitable even for the most fragile populations, who can find benefits in wellness and social relationships. Aikido is also versatile in places of practice because it can be carried out anywhere, although the mat appears to be the best location. In this cross-sectional study, in addition to microbiota analysis, have been collected anthropometric data, biographical data, eating habits, physical activity levels and meditation practice have been evaluated through MEDI, (MEditation Depth Index) and MEDEQ questionnaire (MEditation Depth Questionnaire; Piron, 2008).[36] Data of microbial composition from faecal and salivary material was analyzed in relation to biographical aspects, dietary habits, physical activity and level of meditation depth. Subsequently, the data obtained were compared with data from literature and other disciplines, but only in relation to physical activity, as data in literature on diet and meditation was poor. More specifically, the distributions and fluctuations of microbial composition according to depth levels of Meditation and frequency of Aikido were analyzed. These new data show that some genera tend to change in a statistically significant way in Meditation groups ($p = 0.000\ 1$), while less fluctuations are recorded for Aikido practice levels. Overall, the bacteria enriched in those who practice meditation have previously been associated with improved mental health, behavior and mood. This, in addition to physiological effects, suggests the potentially central role of meditation in mental and physical well-being.[60] Moreover, different bacterial genera differ significantly in various depth levels of Meditation. At the genus level, *Bacteroides*, *Faecalibacterium*, and *Prevotella* are found to be predominant in all groups, but the two first were found to be significantly enriched in Meditation group. *Prevotella* was found to be slightly increased compared to the group with lowest Meditation score (Fig. 6). *Prevotella* is a marker bacterium of intestinal good health, but it is believed that, if not accompanied by good *Bacteroides* and *Akkermansia muciniphila*, it may promote pro-inflammatory processes, infections and dysbiosis.[61] Subjects practicing deep meditation and aikido show that both *Prevotella* and *Bacteroides* are enriched. Scientific evidence supporting this shows that *Prevotella* is deficient in subjects with depressive disorders,[62,63] indicating its connection to the gut-brain axis.[60,64] Also consistent with present data, *Feacalibacterium prausnitzii* and *Roseburia* are increased in moderately active individuals,[64] while vigorous exertion appears to decrease their abundance.[65,66] *Ruminococcus*, a marker of gut dysbiosis, is decreased in exercise intervention studies.[67,68] *Alistipes*, one of the newest genera in *Bacteroidetes* phylum, was found to be decreased in some exercise intervention studies,[69,70] although its role deserves further investigations. Relative abundance of *Enterococcus* spp. is higher after moderate intensity and long duration exercise, when compared to higher intensities and shorter durations.[71] *Megamonas* and *Faecalibacterium* are abundant in meditation practice. *Faecalibacterium* seems to be associated with better quality of life, in fact it decreases under stressful conditions.[72,73] These results may support this

cross-sectional study data showing that this genus increases as meditation practice increases. Furthermore, in subjects who practice aikido and reach high levels of depth meditation, *Roseburia* is enriched as shown in other work on the topic.[59] So, scientific evidence seems to support this cross-sectional study data. In fact, there are works in literature that demonstrate specific changes in gut microbiota of subjects practicing physical activity, martial arts, or meditation and seem in line with these data.[73,74] Specifically, regarding physical activity, studies show that exercise intensity has an influence on *Fimicutes* abundance. Regarding meditation, *Bacteroidetes*, particularly *Prevotella* and *Faecalibacterium*, appear to be enriched in subjects who practice a lot of it. This opens the idea that practicing a Moving Meditation activity, such as Aikido, may result in an eubiotic balance in practitioners. Specifically, *Firmicutes* phylum includes genera producing SCFAs, which are widely recognized for their involvement in crucial processes such as maintaining colonocyte integrity, improving barrier function and increasing mucin expression regulating specific gene expression.[75–79] Particularly, SCFAs (e.g. butyrate, acetate and propionate) exert their physiological activities by binding the G-protein-coupled receptors (GPCRs) and modulating gene expression by inhibiting histone deacetylases (HDACs).[80] GPCRs are cell surface receptor proteins that regulate various physiological and pathological processes, while HDACs are part of the epigenetic regulatory mechanisms that control the expression of genes.[81,82] The defensive cells of microglia in the enteric nervous system (ENS), stimulated by bacterial LPS, trigger an inflammatory response, but this has been shown to be inhibited by acetate and butyrate through the inhibition of HDAC activity and NF-kB activation.[83] Furthermore, scientific evidence supports the notion of an inverse relationship between type and intensity of physical activity and the amount of fecal bile acids influencing *Firmicutes* growth (mainly Clostridia spp.). Besides short-chain fatty acids and bile acids, some recent studies show that gut microbiota also have a key role in neurotransmitter's production such as glutamate, GABA, serotonin, and dopamine.[84,85] These bacteria derived neurotransmitters precursors can arrive to the brain passing the blood-brain barrier and finalize the neurotransmitter synthesis.[86] Moreover, gut bacteria metabolites can interact with enteroendocrine cells and thus acting locally, or transmit to brain via the fast vagus nerve way, to regulate the synthesis and release of neurotransmitters, the so called gut-brain axes.[87,88] On the other hand, it seems that *Bacteroides* play a crucial role in neurotransmitters modulation along the gut-brain axis. It appears to be involved in tryptophan metabolism, a precursor of brain serotonin.[70] So, in general, these observations reinforce the notion that gut bacteria play an active role in metabolic homeostasis and are influenced by practices involving both physical and mental aspects. This study is intended to be a contribution to the characterization of gut microbiota of adults practicing Aikido, a Moving Meditation Discipline. It provides insight into the relative abundances of major bacterial phyla in gut in a sample of 23 participants, suggesting the influence of different Meditation levels and Aikido on the composition of gut microbiota. A results summary of the present work is schematized in Fig. 7 where we may see two different, but complementary situations. On one hand (A), the intensity of exercise seems to be positively correlated with growth in relative abundance of Firmicutes phylum, on the other hand (B) the level of Meditation depth seems to be positively correlated with the increase in relative abundance of Bacteroidetes phylum. Consequently, it follows that Aikido (C), that organically involves a performance in both aspects just described, can balance the Bacteroidetes/Firmicutes ratio, with satisfactory effects on the health and well-being of practitioners.

4.1. Limitations

This cross-sectional study has limitations. First of all, data collected are meager to paint a general picture of gut microbial variation in a population such heterogeneous as that of Aikido practitioners. As the latter is a highly individualized discipline, it can be practiced at different intensities. Nevertheless, in this study, only time of practice was assessed (minutes per week), but it was absent an instrumental assessment of training intensity. Regarding Meditation, the MEDI score concerns only the physical and mental sensations perceived during practice and does not consider the time factor. In addition, although the sample appears to be quite homogeneous, diet and smoking were not considered as possible confounding factors. Despite these limitations, this study represents the first attempt to characterize the effects of Moving Meditation Disciplines, particularly Aikido, on the composition of the gut microbiota and opens new perspectives for future research on exercise strategies involving the mind-body unicum.

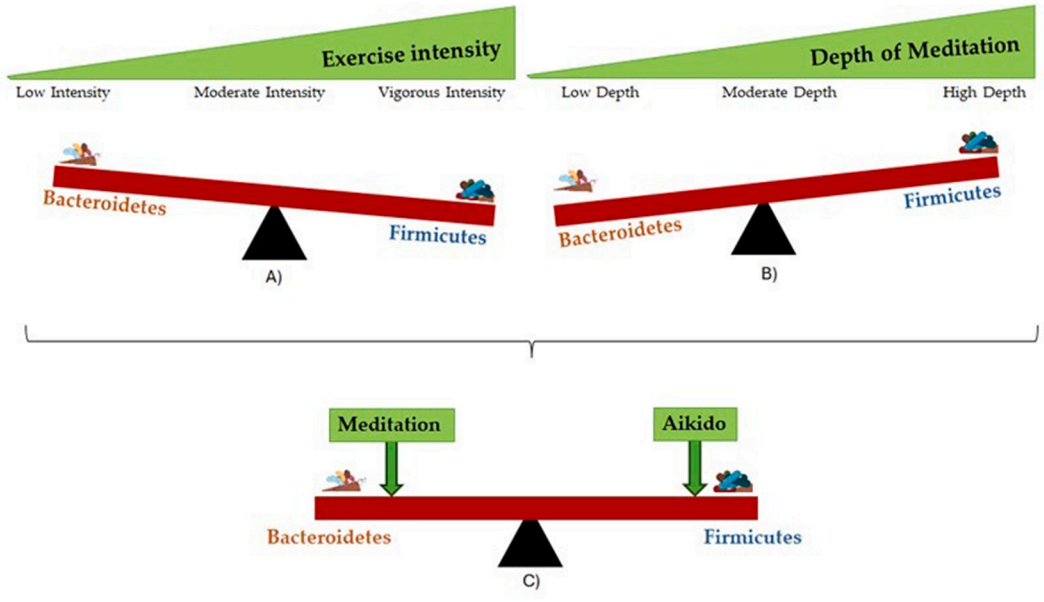


Fig. 7. Firmicutes/Bacteroidetes Ratio in Relation to Moving Meditation Disciplines. Representation of the balance Firmicutes/Bacteroidetes ratio through the practice of Moving Meditation Disciplines.

5. Conclusions

This work represents the first attempt to characterize the gut microbiota of a cohort of adult Aikido practitioners, a Moving Meditation Discipline derived from the integration of ancient Samurai arts with mental and spiritual aspects typical of Japanese world. So, Aikido proposes the systematic union of spiritual and physical components, which can also be proposed at low impact making it suitable or adaptable activity for everyone, even the most fragile populations. Moreover, it can be practiced anywhere and proposed to populations of all ages and conditions, with application to contexts of strong social value such as schools and workplaces. For these reasons, Aikido could represent a discipline of the highest value in health prevention and promotion because of its high sustainability. The results that we obtained showed significant alterations in gut microbiota composition among high-meditation practitioners. In this group, we noticed increased levels of *Bacteroides* and *Faecalibacterium* and a slight rise in *Prevotella*. For what concerns the Aikido level, the moderate-intensity group was linked to an enrichment in Firmicutes. These findings suggest that Aikido, a meditation in movement practice, promotes a balanced Bacteroidetes/Firmicutes ratio, supporting intestinal eubiosis and thus contributing to gut health and well-being.

Considering the current epidemiological scenario of western society, there is a clear and urgent need to spread physical activity as a tool for health promotion and prevention of chronic degenerative diseases. In addition to this, it is increasingly relevant to use simple, sustainable and well-tolerated methods to ensure benefits in a new perspective that considers the body-mind as a unicum. In this sense, Aikido can be effectively employed and considered as a well-being supportive tool in preventing chronic and degenerative diseases. In fact, this present work has shown how this discipline can be helpful and supportive for gut homeostasis, especially when coupled with adequate depth of meditation. The health benefits are massive, and they are not only about cardio-respiratory fitness and muscle function. Instead, they also involve cognitive and mental aspects, with positive consequences on stress control, now a daily component of contemporary society. In fact, meditation has proven to be an excellent and effective tool for improving stress management and individual responses to stressful situations. The present study, with all its critical issues, is meant to be a preliminary work on the interaction between gut microbiota, wellness and Moving Meditation Disciplines. It is desirable that this study would be used as basis for future intervention studies of Moving Meditation in order to verify a role in stress management and chronic disease prevention.

CRediT authorship contribution statement

Veronica Volpini: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Sofia Bricca:** Writing – original draft, Investigation, Conceptualization. **Francesca Ubaldi:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Tehreema Ghaffar:** Writing – review & editing, Visualization, Investigation. **Serena Platania:** Visualization, Investigation. **Federica Valeriani:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Conceptualization. **Vincenzo Romano Spica:** Writing – review & editing, Supervision, Conceptualization.

Ethical approval statement

The study was carried out respecting the principles of the Declaration of Helsinki. The protocol of the study was approved by the Ethical Board of the University of Rome "Foro Italico" (CAR approval number 204/2024).

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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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Meditation in Motion: Sport Type and Meditation Level Shape Gut Microbiota Profiles in Aikido and Tai Chi Practitioners

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Abstract

Mind–body practices integrating movement and meditation, such as Tai Chi and Aikido, have been proposed to influence the gut–brain axis through combined physiological and psychological pathways. However, evidence regarding their association with gut microbiota composition remains limited. This study explored gut microbiota diversity and taxonomic profiles in regular practitioners of Tai Chi and Aikido across different levels of meditation depth. Forty-two adults practicing Tai Chi or Aikido provided fecal samples for 16S rRNA sequencing, and meditation depth was assessed using the Meditation Depth Questionnaire (MEDEQ). Alpha diversity did not differ significantly between groups, although a descriptive trend toward higher diversity with increasing meditation depth was observed. Beta-diversity analyses suggested compositional differences associated with meditation level (ANOSIM R = 0.191, $p = 0.035$), along with an exploratory interaction signal between practice type and meditation depth (ANOSIM R = 0.296, $p = 0.001$). Taxonomic profiling highlighted distinct microbial patterns associated with both practice type and meditation depth. Short-chain fatty acid-associated genera, including *Faecalibacterium* and *Roseburia*, were relatively more abundant in Aikido practitioners with higher meditation scores, whereas Tai Chi practitioners showed higher relative abundances of *Prevotella* and *Collinsella*. Overall, these findings indicate that meditative movement practices are associated with distinct gut microbiota compositional patterns within this cohort. Given the exploratory and cross-sectional design, these results should be interpreted as hypothesis-generating. Future longitudinal studies incorporating functional and clinical outcomes are needed to clarify underlying mechanisms.

Keywords: meditation; sport; gut microbiota; aikido; taichi; gut–brain

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1. Introduction

In an era dominated by digital engagement and increasingly sedentary habits, depression has emerged as one of the most widespread mental health conditions of the 21st century, affecting over 280 million individuals worldwide [1]. Contemporary lifestyles are marked not only by insufficient physical activity but, in specific populations, also by excessive or performance-oriented training [2–4]. Both physical inactivity and excessive exercise have been linked to physiological stress and dysregulation of metabolic, immune, and neuroendocrine pathways, underscoring the importance of balanced, integrative movement-based lifestyle approaches [2–5].

Insufficient physical activity is consistently linked to increased risk of chronic dis-ease, low-grade inflammation, and depressive symptoms [6–9]. Conversely, evidence de-rived largely from competitive athletic populations indicates that prolonged high-intensity training may induce physiological stress, impair immune function, and disrupt gut barrier integrity [10]. Although physical activity per se modulates gut microbiota composition in-dependently of diet across diverse populations [11], more pronounced gastrointestinal disturbances and stress-related microbial alterations are predominantly observed in endurance and performance-oriented athletes exposed to high training loads [12]. These findings highlight that adverse physiological adaptations may arise at both ends of the activity continuum. Despite the well-established benefits of physical activity across the life course, global levels of inactivity remain high, with nearly one-third of adults failing to meet recommended activity levels [1,13–15]. This persistent gap between public health recommendations and real-world behaviors has reinforced interest in accessible, sustainable, and holistic forms of movement that integrate physical and mental dimensions [1,16].

Within this context, the gut microbiota has emerged as a critical mediator connecting lifestyle behaviors with physical and mental well-being [17]. This diverse microbial ecosystem interacts bidirectionally with physical activity, immune function, and metabolic pathways [18]. Active individuals typically exhibit higher microbial richness and more favorable microbial profiles than sedentary counterparts [19,20], contributing to improved gastrointestinal function, reduced systemic inflammation, and enhanced metabolic resilience. However, not all forms of physical activity exert the same influence on the microbiome. Practices integrating intentional movement, breath control, and sustained mental focus—often referred to as meditative movement—may modulate physiological and psychological systems simultaneously, influencing the gut–brain–microbiota axis through distinct mechanisms [21]. Physical activity also impacts the autonomic nervous system, including vagal nerve function, a key component of the gut–brain axis whose dysregulation is closely linked to depression [22,23].

Meditation alone is widely recognized for its benefits in reducing stress, improving emotional regulation, decreasing inflammation, and modulating neuroendocrine pathways [24]. Mindfulness-based practices lower cortisol, support cardiovascular regulation, and attenuate systemic inflammatory markers [25,26]. Stress is a primary mediator of gut microbial composition and intestinal permeability [27]; chronic psychological stress disrupts microbial diversity and promotes dysbiosis, contributing to gastrointestinal and mood disorders [22]. Conversely, meditation and mindfulness may contribute to a more stable gut environment, potentially through reduced inflammatory cytokine signaling and modulation of vagal tone [28,29]. These findings support the hypothesis that practices combining physical movement with meditative engagement may exert synergistic effects on both mental and gut health [28].

Martial arts represent a unique category of mind–body disciplines that integrate physical exercise, breath regulation, focus, and philosophical principles [30]. Tai Chi and Aikido, both traditionally rooted in Eastern philosophies, are soft martial arts characterized by coordinated movement, balance training, controlled breathing, and heightened body awareness [31]. These practices emphasize harmony, proprioception, and mental clarity, positioning them as paradigmatic forms of meditative movement rather than performance-driven exercise [30–32]. Evidence indicates that martial arts practice is associated with improvements in physical fitness, emotional regulation, psychological well-being, and social connectedness, with reductions in anxiety and depressive symptoms reported particularly in non-clinical, community-based populations [31–33].

Tai Chi is a low-impact internal martial art characterized by slow, flowing movements and sustained meditative awareness, with evidence suggesting benefits for mental health,

balance, cardiovascular function, and immune–inflammatory regulation [34–38]. The available literature further indicates that Tai Chi and other martial arts may reduce depressive symptoms, particularly in the short term, with mental health benefits partly mediated by social connectedness arising from group-based practice settings, as shown by mediation analyses in intervention studies [36–40].

Within the spectrum of meditative martial arts, Aikido represents a distinct Japanese discipline emphasizing harmony, circular and fluid movements, breath regulation, and embodied awareness [41]. Often described as "meditation in action," Aikido cultivates heightened attention, centeredness, and psychological calm during practice [42]. Although less extensively studied than Tai Chi, Aikido shares core elements of mind–body integration and may exert comparable influences on mental and physiological pathways, including stress-responsive and immune-related mechanisms [43–45].

The metabolic intensity of martial arts varies considerably: Tai Chi ranges from 1.5 to 3.0 METs (Metabolic Equivalent of Task) (light-to-moderate intensity), slower-paced Aikido averages around 5.3 METs (moderate intensity), whereas combat sports such as judo, karate, or kickboxing reach 10.3 METs (vigorous intensity) [46]. Unlike high-intensity competitive sports, meditative martial arts integrate moderate physical activity with mindfulness and self-regulation, potentially offering unique benefits for mental health [32,38,40,47–49]. By operating within light-to-moderate intensity ranges, they are consistent with international physical activity guidelines and may represent sustainable, accessible forms of movement across the life course [14,15,32].

Nevertheless, empirical evidence on gut microbial profiles in individuals practicing meditative martial arts remains limited [50]. While extensive research has examined the gut microbiota of competitive athletes—demonstrating sport-specific microbial adaptations driven by training intensity and physiological load [5,51–55]—non-competitive, meditative martial arts remain underexplored. Studies demonstrate that the type of sport, intensity of training, and physiological load differentially shape gut microbial profiles, with endurance, strength, and combat sports each presenting distinct signatures [5,54]. These findings highlight the adaptive capacity of the microbiota to the metabolic, neuroendocrine, and inflammatory demands of training [53–55]. However, most existing research has focused on competitive athletes engaged in high-intensity or performance-driven activities, leaving an important gap regarding non-competitive, meditative martial arts such as Tai Chi and Aikido [56,57]. Their unique combination of moderate physical activity, mindfulness, and autonomic regulation may influence the gut microbiota through mechanisms distinct from those observed in performance-oriented sports [54–57].

The present study, therefore, investigates gut microbiota composition in individuals practicing Tai Chi and Aikido, to explore whether these meditative movement practices are associated with distinct microbial profiles in relation to both sport type and depth of meditative engagement. By focusing on a population that integrates movement and meditation simultaneously, this exploratory study seeks to address an important gap in current microbiome research and to generate hypotheses for future mechanistic and longitudinal investigations.

2. Materials and Methods

2.1. Study Design and Ethical Approval

The research protocol received approval from the Institutional Review Board at the University of Rome "Foro Italico" (approval number CAR 204/2024). The study was conducted in accordance with the principles of the Declaration of Helsinki. During the recruitment process, investigators explained the aims of the study, its procedures, and

the ethical safeguards in place, including data anonymity and confidentiality. Before participation, all subjects provided written informed consent.

2.2. Participants and Recruitment

A total of 53 adult practitioners of Tai Chi and Aikido were invited to participate after their regular training sessions. Of these, 46 agreed to take part and completed all study procedures, including informed consent, questionnaire completion, and biological sample collection. Four samples were excluded from the final analysis due to protocol non-compliance (e.g., improper labeling or delayed sample submission). Consequently, the final analytical sample consisted of 42 participants, as shown in Figure 1. Inclusion criteria included regular practice of Tai Chi (Tai Chi group) or Aikido (Aikido group), willingness to provide biological samples and complete questionnaires, and absence of antibiotic or probiotic use or gastrointestinal procedures within the previous three months. All participant data were anonymized using a non-identifiable alphanumeric code, consistently applied to biological samples and questionnaires. Participants were adults within an age range of 18 years and older (including individuals > 65 years), and sex distribution is summarized in Table 1. All inclusion and exclusion criteria, including antibiotic or probiotic use, recent gastrointestinal procedures, chronic diseases, pregnancy, and other medical conditions potentially affecting gut microbiota composition, were applied during the recruitment phase, prior to participant enrollment and biological sample collection.

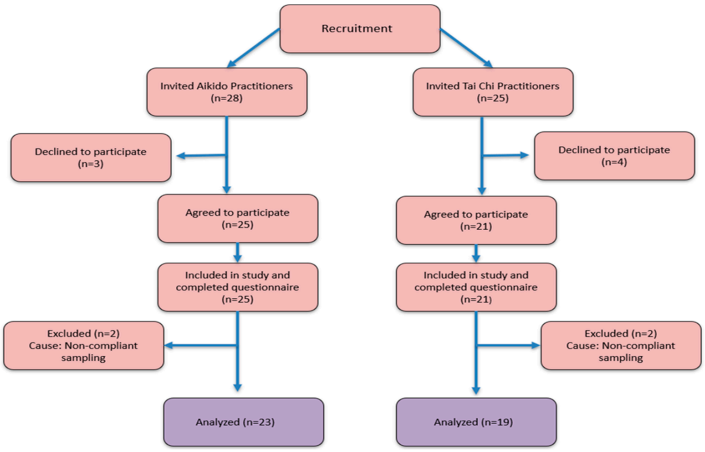


Figure 1. Study flowchart of participant enrollment and analysis. Diagram showing recruitment of 53 invited participants, exclusions for non-compliant sampling, and final inclusion of 42 Tai Chi and Aikido practitioners with complete questionnaire and fecal microbiota data.

Table 1. Socio-demographic characteristics of Tai Chi and Aikido participants.

Variable	Value Aikido	Value Taichi	*p*-Value
Gender			
female	9 (39.13%)	14 (73.68%)	0.03
male	14 (60.87%)	5 (26.31%)	
Age			
18–30	1 (4.17%)	1 (5.26%)	0.29
31–65	20 (83.33%)	12 (63.16%)	
>65	3 (12.5%)	6 (31.58%)	

Table 1. *Cont.*

Variable	Value Aikido	Value Taichi	*p*-Value
Educational level			
Middle school graduation	1 (4.17%)	0 (0.00%)	0.36
High school degree	9 (37.50%)	6 (40.00%)	
Bachelor's degree	1 (4.17%)	2 (13.33%)	
Master's degree	9 (37.50%)	7 (46.67%)	
PhD	4 (16.67%)	0 (0.00%)	
Smoking			
No	16 (66.67%)	14 (73.68%)	0.74
Yes	8 (33.33%)	5 (26.32%)	
Smoking frequency			
At least once/day	6 (75.00%)	2 (40.00%)	0.42
4–6 times/week	1 (12.50%)	2 (40.00%)	
Maximum 3 times/week	1 (12.50%)	1 (20.00%)	
BMI			
Normal weight	17 (70.83%)	12 (63.16%)	0.50
Underweight	0 (0.00%)	1 (5.26%)	
Overweight	6 (25.00%)	6 (31.58%)	
Obese	1 (4.17%)	0 (0.00%)	
Diet			
No specific diet	10 (41.67%)	5 (26.32%)	0.3
Mediterranean	8 (33.33%)	12 (63.16%)	
Vegetarian	3 (12.50%)	1 (5.26%)	
Requested by physician	1 (4.17%)	0 (0.00%)	
Caloric restriction	1 (4.17%)	0 (0.00%)	
Gluten free	0 (0.00%)	1 (5.26%)	
Other	1 (4.17%)	0 (0.00%)	
Alcohol consumption			
No alcohol	0 (0.00%)	3 (15.79%)	0.20
Often (>5 times/month)	11 (45.83%)	6 (31.58%)	
Sometimes (3–4 times/month)	10 (41.67%)	7 (36.84%)	
Occasionally (1–2 times/month)	3 (12.50%)	3 (15.79%)	
Aikido practice			
<300 min/week	14 (58.33%)	9 (47.37%)	0.50
>300 min/week	10 (41.67%)	10 (52.63%)	
General physical activity			
Partially active	4 (16.67%)	6 (31.58%)	0.29
Physically active	20 (83.33%)	13 (68.42%)	
MEDI score			
Low (0–60)	10 (41.67%)	17 (89.47%)	0.005
Medium (60–80)	7 (29.17%)	1 (5.26%)	
High (80–120)	7 (29.17%)	1 (5.26%)	
Meditation time			
<60 min/week	13 (54.17%)	6 (31.58%)	0.13
>60 min/week	11 (45.83%)	11 (57.89%)	
Not available	0 (0.00%)	2 (10.53%)	

2.3. Biological Sample Collection and Handling

Each participant received a fecal swab (Copan Italia S.p.A., Brescia, Italy) along with standardized instructions for fecal sample collection. A single fecal sample was collected from each participant as part of this cross-sectional study design. Participants were asked to collect a single fecal sample on a designated day and to submit the swab within two hours of collection. While a strict circadian collection window was not imposed, participants were instructed to collect samples under consistent daily conditions and to avoid collection immediately following intense physical activity. Samples were stored at 4–8 °C in a refrigerated container and transported to the laboratory at the University of Rome "Foro Italico" within 24 h, where they were processed according to a previously validated protocol for DNA extraction from fecal material.

2.4. Questionnaire

Participants were asked to fill out a questionnaire on the EU survey platform, which collected details about their age, gender, weight, height, and any specific dietary practices they might follow (such as vegetarian or vegan diets). It was emphasized that providing this information would not result in the exclusion of any participants. Furthermore, the presence of ongoing chronic illnesses, pregnancy, food sensitivities, concurrent infections (along with the use of antibiotics and probiotics), or gastrointestinal surgical procedures within the last three months were considered as exclusion criteria. The 9-item questionnaire created by Martínez-González et al. and the abbreviated version of the International Physical Activity Questionnaire (IPAQ) were utilized to evaluate participants' eating habits and typical physical activity levels [58,59]. In addition, a 35-item evaluation tool known as the Meditation Depth Questionnaire (MEDEQ) was implemented to assess the intensity of meditation engaged in by each participant [60]. The opening questions of this questionnaire focus on the techniques and duration of meditation, covering both the number of years practiced and the minutes spent in each session. The latter section consists of 30 items aimed at gathering insights about the physical and mental experiences encountered by subjects during meditation. This information is then leveraged to calculate the MEDI, a quantitative metric for meditation depth. The MEDI and MEDEQ tools were both devised by Piron et al. [60]. For every question, participants were asked to indicate their degree of agreement on a scale from 0 (indicating no agreement) to 4 (indicating strong agreement). The overall score was derived by adding up the factors identified as supportive of meditation specifically factors 3, 4, 6, 7, 8, 9, 11, 12, 15, 16. The final score is reached by summing up the scores for factors 16, 17, 19–30, and items 1, 4, 9, 12, 13, and 18, which are inversely linked to meditation. It is important to highlight that the total score does not pertain to the time element, but rather to the physical and mental sensations experienced during meditation. Although the Meditation Depth Questionnaire (MEDEQ) and the International Physical Activity Questionnaire–Short Form (IPAQ-SF) have been validated in their respective original populations, they have not been specifically validated in practitioners of meditative martial arts such as Tai Chi or Aikido. In the present study, these instruments were therefore used as exploratory tools to characterize relative differences in meditation depth and general physical activity levels within the study cohort, rather than for diagnostic or comparative purposes across populations.

2.5. Data Collection

Given the exploratory and cross-sectional nature of the study, a formal a priori sample size calculation was not performed to power specific hypothesis-testing comparisons. The final sample size was determined by feasibility and participant availability during the recruitment period. Any previously reported power considerations based on paired

designs have been removed, as they were not appropriate for the unpaired study design. Participants were recruited after an invitation presented at the end of class. Those who agreed to participate received the study materials on pre-scheduled days. The materials included an information letter, an informed consent form, written instructions, test tubes for fecal sample collection, corresponding labels, and a link to access the questionnaire. The fecal samples were collected separately, adhering to detailed guidelines, and were kept refrigerated until they were delivered on designated days and times. To maintain anonymity, each participant was assigned a code made up of the first three letters of the mother's name and the father's birth year (e.g., FRA1967), which remained unknown to the researchers. The identification code was affixed to each tube using the supplied labels and included in the questionnaire, allowing for cross-analysis of the data. The questionnaire, which contained both open-ended and closed-ended questions, was distributed through a link on the EU Survey platform, the official European Commission tool for conducting online surveys.

2.6. DNA Extraction from 16S rDNA Amplicon

A fecal swab (Copan Italia S.P.A., Brescia, Italy) was given to each participant, along with detailed instructions for stool sample collection. Participants were told to submit their samples on a specified date, within two hours of collection. The samples were kept at 4–8 °C in a refrigerated container and transported to the laboratory at the University of Rome "Foro Italico" within 24 h. Upon receipt, fecal samples were weighed, and DNA was extracted following a validated protocol for isolating microbial DNA from fecal material. The DNA was then purified and normalized before sequencing. Amplicon libraries were created targeting the V1–V3 region of the 16S rRNA gene, adhering to the 16S Metagenomic Sequencing Library Preparation Guide (part# 15044223 Rev. A; Illumina, San Diego, CA, USA) [61,62]. Tagged polymerase chain reaction (PCR) products were generated through a two-step PCR process using unique barcoded primer pairs. In this approach, the target primers containing adapters were employed in the first PCR to amplify the target gene (16S rDNA), and the resulting product was used in the second PCR with primers that included barcodes for individual sample identification. Each amplification reaction comprised a total volume of 25 µL, including 12.5 µL of KAPA HiFi Hot Start Ready Mix (Roche, Pleasanton, CA, USA), 5 µL of each primer (1 µM), and 2 µL of template deoxyribonucleic acid (DNA). Reactions were conducted on a Techne® TC-PLUS thermal cycler (VWR International, LLC, Radnor, PA, USA).

2.7. Illumina Sequencing and Bioinformatics Analysis

The amplification is performed and 5 µL of PCR product from every reaction was analyzed through agarose gel electrophoresis (1%) to verify amplification. The final concentration of the purified DNA amplicon was measured using the Qubit PicoGreen dsDNA BR Assay Kit (Invitrogen, Grand Island, NY, USA). Libraries were prepared following the MiSeq Reagent Kit Preparation Guide (Illumina, San Diego, CA, USA) and utilized the iSeq100 sequencing platform (Illumina, San Diego, CA, USA). Raw sequencing data were processed with a customized bioinformatics pipeline based on the Galaxy platform, incorporating quality control and taxonomic assignment tools. Initial quality evaluation of FASTQ files was carried out using FASTA/Q Information Tools and Mothur. Reads were filtered to remove low-quality sequences (length < 200 nt), ambiguous base calls, and chimeric sequences. High-quality sequences were grouped into operational taxonomic units (OTUs) at 97% sequence identity, employing a de novo clustering method. Representative sequences from each OTU were classified taxonomically using the Ribosomal Database Project (RDP) Classifier (version 2.5), which assigns taxonomic ranks through a naïve

Bayesian algorithm. OTUs identified at 97% similarity were regarded as species-level units, while 95% similarity was applied for genus-level classifications. Furthermore, sequencing reads were concurrently examined using the Illumina BaseSpace 16S Metagenomics App (version 1.0.1), which employs an alternative taxonomic classification pipeline based on the curated May 2013 release of the Greengenes reference database. Only taxes with a relative abundance exceeding 0.1% were included in subsequent statistical analyses.

2.8. Statistical Analysis

Meditation depth was assessed using the MEDI score and, for descriptive purposes, participants were categorized into three levels (low, medium, high). The MEDI reflects subjective physical and mental experiences during practice rather than meditation duration. Given the limited size and imbalance of some meditation-level strata, analyses involving meditation categories were interpreted exclusively as exploratory. Gut microbiota relative abundances were calculated at the phylum and genus levels using an OTU-based approach at 97% sequence similarity. Taxonomic assignment was performed using the RDP classifier and the Greengenes reference database. Very low-abundance phyla (<0.1% across all samples) were excluded to reduce data sparsity. Alpha diversity (Shannon index and evenness) and beta diversity were computed using PRIMER7. Normality of alpha-diversity distributions was assessed using the Shapiro–Wilk test. Group comparisons were conducted using parametric or non-parametric tests as appropriate (independent samples t-test, Mann–Whitney U test, or Kruskal–Wallis test). Genus-level richness was estimated using a Chao1-like index as an exploratory complement [63]. Beta-diversity patterns were explored using principal coordinate analysis (PCoA), and differences in community composition were assessed using analysis of similarity (ANOSIM). Given the exploratory design and baseline imbalances in sex distribution and MEDI score, these variables were considered during interpretation rather than used for confirmatory adjustment. All statistical analyses were performed using PRIMER-e Ltd. (version 7.), Python (version 3.10) with the SciPy library, and IBM SPSS Statistics version 25. Statistical significance was set at $p < 0.05$. For analytical purposes, participants were categorized according to practice type (Tai Chi vs. Aikido) and sex (male vs. female). Sex-stratified analyses were conducted within each practice group for descriptive comparison only.

3. Results

3.1. Participant Information and Study Design (General Characteristics of the Subjects)

We collected a total of 42 fecal samples from adult practitioners of two meditative martial arts, Tai Chi and Aikido (Table 1). Both practices integrate structured physical movement with meditative components. Descriptive analyses were performed for anthropometric, sociodemographic, and lifestyle variables. Body mass index (BMI) was calculated as body weight (kg) divided by height squared (m^2). For analytical purposes, participants were categorized according to two independent factors: (i) type of meditative movement practice (Tai Chi vs. Aikido) and (ii) sex (male vs. female). Analyses examined the main effects of practice type and meditation depth, with additional sex-stratified comparisons performed within each practice group. The term "exercise + gender" refers exclusively to these sex-stratified analyses and does not represent a separate or combined experimental group. Data were collected on age, sex, BMI, educational level, smoking and alcohol habits, dietary patterns, physical activity level, meditation score, and practice level. Overall, the two groups were largely comparable across most variables; however, significant differences were observed in sex distribution and MEDI score (Table 1). Physical activity was quantified in metabolic equivalent of task (MET) units. Data are reported as mean ± standard

deviation (SD), and no significant differences were detected for the remaining variables based on t-test comparisons.

3.2. Alpha Diversity Indices

Alpha diversity analysis (Shannon and Simpson indices) was evaluated across the categories of Sport, Meditation levels, Physical activity levels, Gender and BMI using *t*-test, Mann–Whitney U test and Kruskal–Wallis test. No statistically significant differences were found in the Shannon or Simpson indices when comparing the groups ($p > 0.05$ for all comparisons). A marginally significant trend was noted for the Shannon index when examining different sports, implying a potential variation between Taichi and Aikido, although this did not reach the level of statistical significance. No significant differences in alpha diversity were observed between Aikido and Tai Chi groups (Shannon $p = 0.103$; Simpson $p = 0.38$), although the Aikido group tended to exhibit higher diversity (Figure 2A). Boxplots depicting the diversity indices highlighted these trends, with the Shannon index spanning from approximately 1.8 to 2.25 among the groups. The Simpson index showed relative stability, ranging from about 0.71 to 0.81. When considering additional factors, stratified by Meditation level, no significant trends were observed in alpha diversity (Kruskal–Wallis test; Shannon: $p = 0.58$; Simpson: $p = 0.48$). Diversity indices remained comparable across low, medium, and high meditation groups. For Taichi + Meditation levels 1 and 2, only one sample was available for each ($n = 1$), which limited the statistical power in these specific subgroups (Figure 2B). Moreover, no significant differences were found, the visual patterns were visually observed that the type of sport might affect diversity indices. No significant differences were detected between male and female participants (Shannon: $p = 0.29$; Simpson: $p = 0.47$), indicating that gender did not influence gut microbial diversity in this cohort (Figure 2C). Analysis of diversity across Physical Activity levels yielded no significant differences (Shannon: $p = 0.89$; Simpson: $p = 0.98$); both lower and higher PA groups displayed overlapping ranges of diversity indices (Figure 2D). Similarly, BMI categories like underweight, normal weight, overweight (Kruskal–Wallis test; Shannon: $p = 0.97$; Simpson: $p = 0.85$) did not show significant effects on alpha diversity (Figure 2E).

Overall alpha diversity (Shannon and Simpson) was not significantly different between Aikido and Tai Chi participants, although a tendency toward higher diversity was observed in the Aikido group (Mann–Whitney U test: Shannon $p = 0.103$; Simpson $p = 0.38$). Among other variables, higher alpha diversity values were observed across increasing meditation levels. Given the very small size of some meditation-level subgroups, these observations are presented for descriptive purposes only and should be interpreted with caution. As an exploratory complement to Shannon and Simpson indices, genus-level richness was estimated using a Chao1-like index (mean $\pm$ SD: 189.7 $\pm$ 50.3; range: 103.6–287.5). Chao1 estimates were consistent with patterns observed for the other alpha-diversity indices and did not materially affect the interpretation of the results.

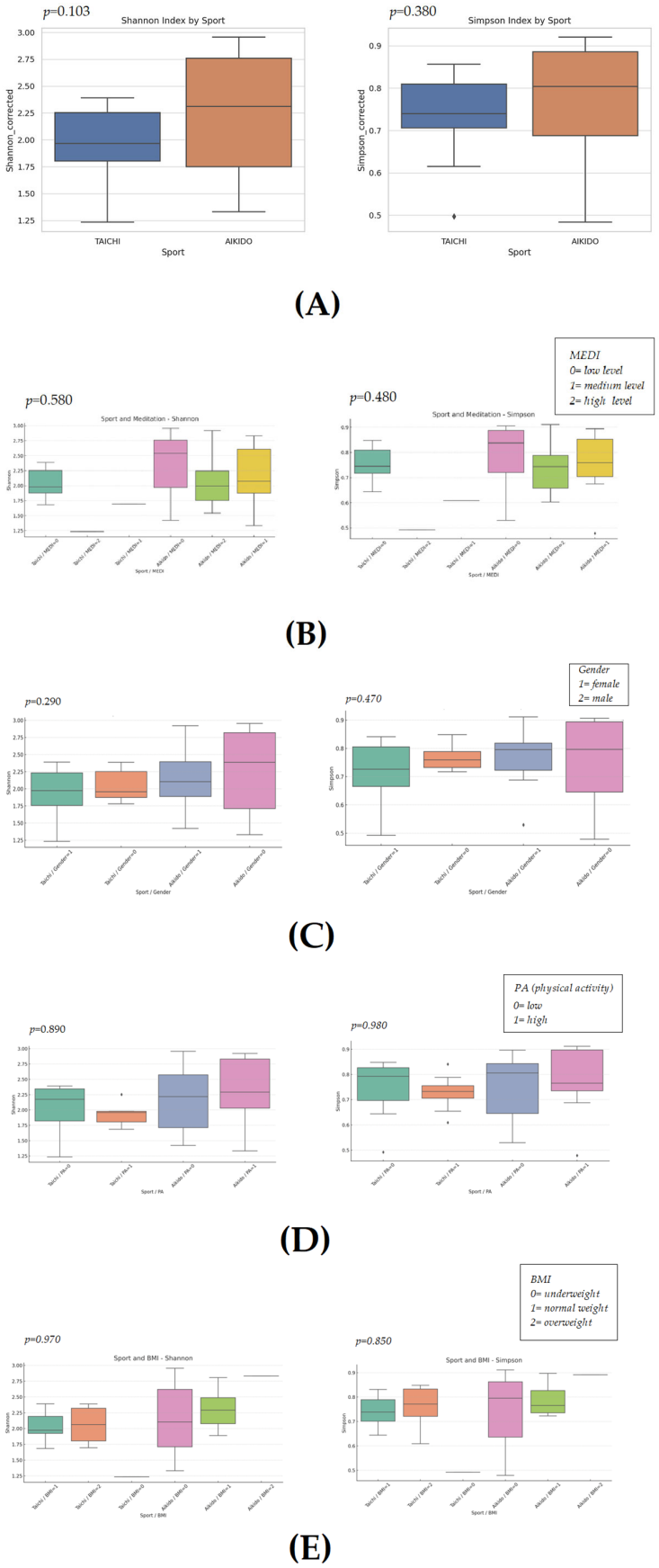


Figure 2. (**A**). Alpha diversity by sport type. Boxplots of Shannon and Simpson indices comparing Tai Chi and Aikido practitioners. Aikido tended toward higher diversity, although differences were

not statistically significant. (**B**). Alpha diversity by meditation level. Boxplots of Shannon and Simpson indices across low, medium, and high meditation groups. No significant differences were observed, but higher meditation levels suggested a trend toward increased diversity. Meditation-level subgroups include highly imbalanced sample sizes (including $n = 1$); results are shown for descriptive purposes only. (**C**). Alpha diversity by gender. Boxplots of Shannon and Simpson indices separated by male and female participants. Diversity measures were comparable across genders. (**D**). Alpha diversity by sport level. Boxplots of Shannon and Simpson indices stratified by reported intensity or level of practice. No significant differences were detected. (**E**). Alpha diversity by BMI. Boxplots of Shannon and Simpson indices for normal weight, overweight, and underweight categories. Indices were consistent across BMI groups.

3.3. Beta Diversity and Compositional Differences (PCoA)

Beta diversity was assessed using Bray–Curtis dissimilarity and represented through Principal Coordinates Analysis (PCoA). The initial two PCoA axes accounted for 56.85% and 15.78% of the overall variation, respectively. Clear separation patterns were noted between the Aikido and Tai Chi groups, indicating differences in their microbiota composition (Figure 3). Confidence ellipses (95%) were incorporated to illustrate the dispersion at the group level. In this group of Aikido and Taichi practitioners, the level of Meditation was found to be the most significant factor impacting the structure of the gut microbial community. Notable differences in beta diversity were detected across varying Meditation levels (R = 0.191–0.240, $p < 0.05$), with the strongest exploratory signal observed for the Sport × Meditation interaction (R = 0.296, $p = 0.001$), indicating that the microbiota response to meditation is influenced by the type of sport practiced. However, this finding should be interpreted with caution due to the very small size of some meditation-level strata and is intended as hypothesis-generating. A slight interaction between Sport and Gender was also identified (R = 0.091, $p = 0.049$). On the other hand, Sport type alone, BMI, Physical Activity level, and Gender did not have a significant impact on beta diversity. These results underscore the potential influence of meditative practices on gut microbiota within this active cohort.

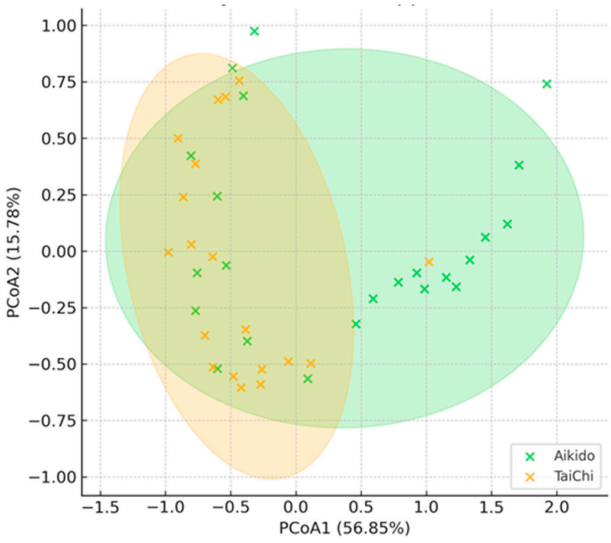


Figure 3. Beta diversity by sport type. Principal Coordinates Analysis (PCoA) of Bray–Curtis distances showing partial separation of Tai Chi and Aikido microbiota compositions with 95% confidence ellipses.

At the level of genus (Figure 4a), PCoA1 and PCoA2 accounted for 67.54% and 10.75% of the overall variance, respectively. Samples from Aikido and Tai Chi showed some degree of separation, although there was also considerable overlap. The primary gen-

era influencing this distinction included: *Prevotella* (more abundant in Tai Chi); *Bacteroides* (more abundant in Aikido); along with *Sutterella*, *Ruminococcus*, *Collinsella*, *Dialister*, and *Lachnospira*. This indicates that the community structure at the genus level varies moderately between the two groups, with certain taxa responsible for driving these variations. At the phylum level (Figure 4b), PCoA1 and PCoA2 represented 79.08% and 17.92% of the variance. A more distinct separation between Aikido and Tai Chi samples was noted. The key contributors to this separation included *Firmicutes* (more prevalent in Taichi) and *Bacteroidetes* (more prevalent in Aikido), along with additional contributions from *Proteobacteria*, *Actinobacteria*, and *Cyanobacteria*. These results highlight that the composition of gut microbiota at the phylum level more effectively differentiates between practitioners of Aikido and Tai Chi than at the genus level. Collectively, these analyses reveal that both genus and phylum level compositions of microbiota differ between Aikido and Tai Chi groups, with the most significant variations linked to changes in the relative abundance of *Firmicutes* and *Bacteroidetes*.

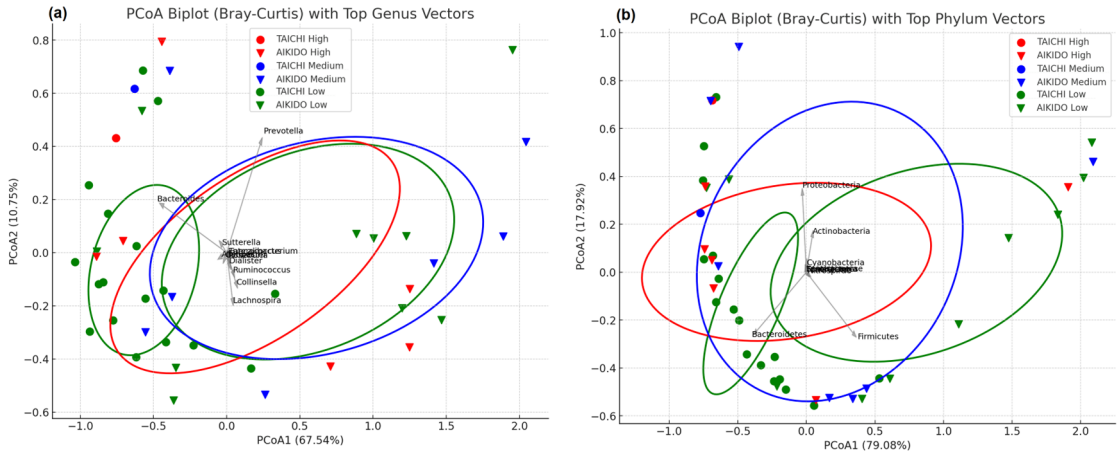


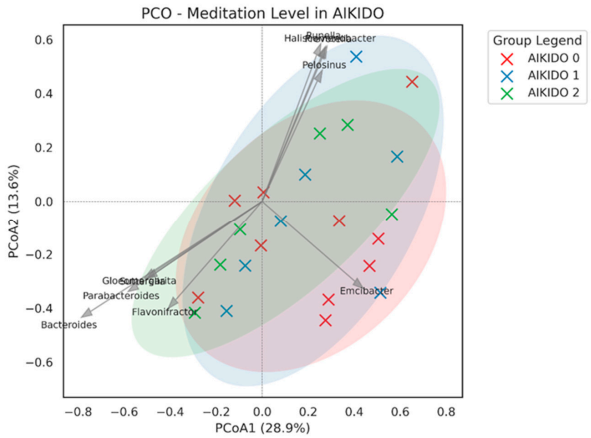
Figure 4. Beta diversity at genus and phylum levels. (**a**). PCoA plots based on Bray–Curtis distances at the genus level, highlighting differences in microbial composition between Tai Chi and Aikido participants. (**b**). PCoA based on Bray–Curtis distances at the phylum level, showing the contribution of dominant phyla (e.g., *Firmicutes* and *Bacteroidetes*) to sample separation.

The Analysis of Similarities (ANOSIM) indicated notable differences in the composition of gut microbiota across varying levels of meditation and between groups defined by sport type and meditation level (Table S1). The most pronounced separation was found in the combined TYPE + LEVEL MEDI comparison (R = 0.296, p = 0.001), suggesting that the type of sport and the level of meditation together influence distinct microbial profiles. Additionally, significant differences were noted in both gender-specific comparisons and overall meditation level assessments.

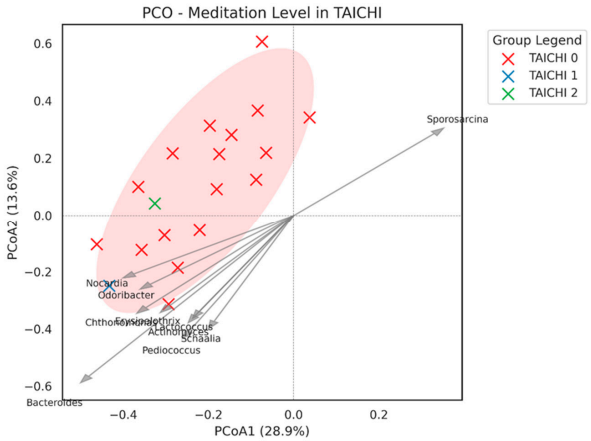
3.4. Gut Microbiota Composition Differences Based on Meditation Levels & Gender

PCoA plots categorized by meditation levels (Low, Medium, High) within the TaiChi and Aikido groups (Figure 5A,B) exhibited observable patterns of variation: Participants with Low, Medium, and High meditation levels displayed distinct yet overlapping clusters in both groups. In the Aikido group (Figure 5A), a more gradual distinction in microbial communities across meditation levels 0, 1, and 2 was found, with some overlap between the groups. However, a slight trend toward differentiation in communities along the PCoA axes was noticeable. The first two axes similarly accounted for 28.9% and 13.6% of the variance. Important genera contributing to this distinction included *Bacteroides*, *Parabacteroides*, *Flavonifractor*, and *Pelosinus*, which could indicate functional microbial adaptations associated with meditation practice in Aikido. In the Taichi group (Figure 5B), the PCO indicated

a partial separation of samples based on meditation level. Samples from meditation level 0 (those not engaging in meditation, marked in red) formed a distinct and tightly clustered group, while samples from higher meditation levels (levels 1 and 2) appeared more spread out among the first two principal coordinates, accounting for 28.9% and 13.6% of the total variance, respectively. In the Tai Chi group (Figure 5B), the PCoA indicated a partial separation of samples according to meditation level. Importantly, vectors for taxa such as *Sporosarcina*, *Odoribacter*, and *Bacteroides* significantly influenced the ordination space, underscoring specific microbial changes linked to meditation level. These findings were corroborated by ANOSIM analyses. Overall, meditation level significantly influenced gut microbial beta diversity across the entire cohort (R = 0.191, $p = 0.035$). Additionally, a strong interaction between sport and meditation was identified (R = 0.296, $p = 0.001$), implying that the effect of meditation level on microbiota composition may differ between Taichi and Aikido practices.



(**A**)



(**B**)

Figure 5. (**A**). Beta diversity by meditation level in Aikido. PCoA biplots of Bray–Curtis distances illustrating clustering of microbial communities across low, medium, and high meditation subgroups, with Bacteroides and Parabacteroides contributing to variation. (**B**). Beta diversity by meditation level in Tai Chi. PCoA biplots showing overlapping clusters with some differentiation between non-meditators and higher meditation groups, with taxa such as Sporosarcina and Odoribacter influencing ordination.

The combined effect of meditation levels in taichi and aikido together shows the leading taxa, such as *Prevotella*, *Bacteroides*, *Ruminococcus*, and *Collinsella*, were responsible for this variation, with *Prevotella* typically linked to higher PCoA1 values and *Bacteroides* connected to lower PCoA1 values. At the genus level, taxa like *Prevotella* and *Bacteroides*

continued to be major contributors to the variation observed along the PCoA axes. PCoA1 and PCoA2 accounted for 67.54% and 10.75% of the total variance (Figure 6a), respectively, consistent with genus-level patterns showing a good consistency. Moreover, a Spearman correlation analysis conducted between the MEDI score and the relative abundance of important bacterial genera did not show any significant relationships ($p > 0.05$), the spread of the ellipses implied that meditation levels might affect microbiota structure, although the separation was not absolute. In both Aikido and TaiChi participants (Figure 6b), gender-based PCoA biplots indicated a degree of separation between male and female participants: The first two PCoA axes accounted for 67.54% (PCoA1) and 10.75% (PCoA2) of the variance. PCoA biplots indicated some degree of gender-based separation, with partial clustering of male and female samples, particularly along PCoA1. In both groups, *Prevotella* and *Bacteroides* were the primary genera that contributed to the variation along PCoA1. Other genera, including *Ruminococcus*, *Collinsella*, and *Dialister*, also played a role in the distribution of samples. Ellipses illustrated some differences in clustering between male and female participants, though there were also overlaps, suggesting a minimal impact of gender on microbiota composition. While gender appeared to contribute modestly to microbiota composition, the observed differences were not absolute; confidence ellipses for male and female participants overlapped substantially. These findings suggest that gender might play a secondary role relative to the type of physical activity practice Aikido vs. Tai Chi in shaping gut microbiota in this cohort.

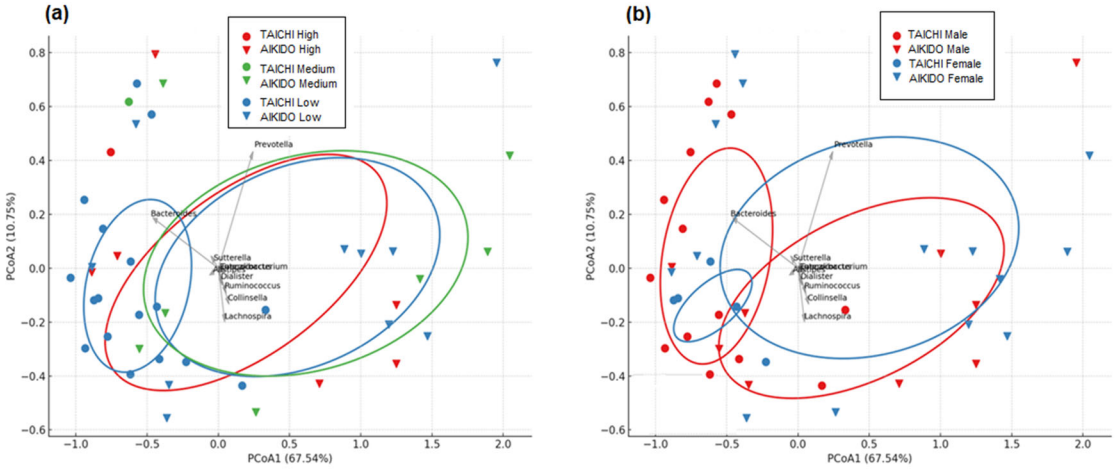


Figure 6. Beta diversity by meditation level and gender. (**a**). PCoA biplots combining meditation scores with sports category. (**b**). PCoA biplots combining meditation scores and gender, indicating partial clustering of samples with Prevotella and Bacteroides as key taxa.

3.5. Genus & Phylum Level Microbiota Composition and Differences

A heatmap of genus-level mean abundance (normalized using Z-scores) was created to assess the differences in gut microbiota composition between the Aikido and Tai Chi groups, among the 503 genera detected and the top 50 most abundant genera were visualized in the heatmap (Figure 7). The heatmap featured a diverse array of bacterial genera, including significant taxa such as *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus*, *Alistipes*, *Sutterella*, *Collinsella*, *Roseburia*, *Blautia*, and *Lactobacillus*, among others. Distinct patterns of genus abundance were identified between the groups: certain genera exhibited greater relative abundance in one group as opposed to the other (positive versus negative Z-scores). For instance, genera like *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus*, and *Alistipes* generally demonstrated increased abundance in one group, whereas genera such as *Lactobacillus*, *Barnesiella*, and *Collinsella* were found to be more abundant in the opposing group. Z-score normalization facilitated the visualization of relative abundance

differences regardless of the actual abundance levels. The vertical axis displays the genera, while the horizontal axis represents the Aikido and Tai Chi groups. The color intensity denotes the Z-score of the mean genus abundance. Shades of red (positive Z-scores) signify a higher-than-average abundance in the respective group, whereas shades of blue (negative Z-scores) indicate a lower-than-average abundance. A Z-score of 0 reflects the average abundance across both groups. To evaluate the differences in gut microbiota composition at the genus level between practitioners of Aikido and Tai Chi, a non-parametric Mann–Whitney U test was conducted on the normalized relative abundances of each identified genus. The resulting *p*-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) procedure. Among the 503 genera that were assessed, five displayed statistically significant differences between the two groups (FDR < 0.05): *Thioalkalivibrio*, *Pediococcus*, *Faecalibaculum*, *Butyrivibrio*, and *Rhodanobacter*. It is important that *Thioalkalivibrio*, *Pediococcus*, and *Faecalibaculum* showed highly significant disparities (FDR-adjusted $p < 1.0 \times 10^{-6}$).

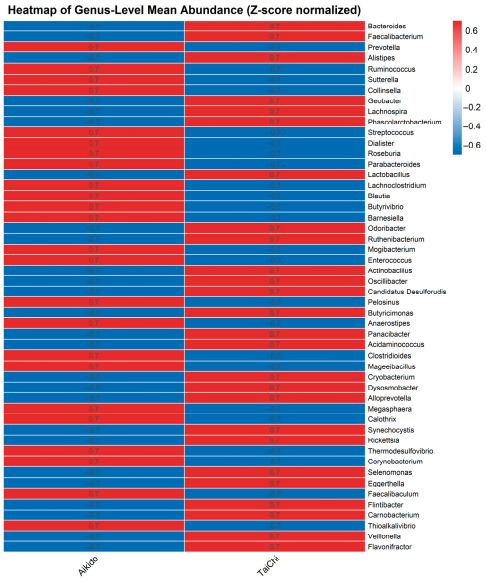


Figure 7. Genus-level abundance heatmap. Heatmap of normalized mean abundance (Z-scores) across 50 most abundant bacterial genera in Tai Chi and Aikido participants. Differences included *higher Faecalibacterium* and *Roseburia* in Aikido and greater *Collinsella* in Tai Chi.

Additionally, a heatmap of phylum-level mean abundance (normalized using Z-scores) was generated to examine the gut microbiota composition between the Aikido and Tai Chi groups (Figure 8). This normalization enables the comparison of patterns among genera with varying absolute abundances, and a total of 30 phyla detected. Overall, the heatmap underscores the compositional differences in gut microbiota profiles of the Aikido and Tai Chi participants. Overall *Firmicutes* and *Bacteroidetes* dominate both groups, but Aikido samples show a stronger relative abundance (brighter signal) of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* while Taichi samples have a generally lower and more uniform profile. Other phyla (many on the right of the heatmap) are essentially absent (dark purple) in both groups. At the phylum level, five phyla displayed statistically significant differences between the groups (FDR < 0.05): *Armatimonadetes*, *Candidatus Gracilibacteria*, *Planctomycetes*, *Nitrospirae*, and *Fusobacteria*. Notably, *Armatimonadetes* and *Candidatus Gracilibacteria* revealed highly significant differences (FDR-adjusted $p < 1.0 \times 10^{-4}$), indicating distinct ecological patterns associated with the two movement practices.

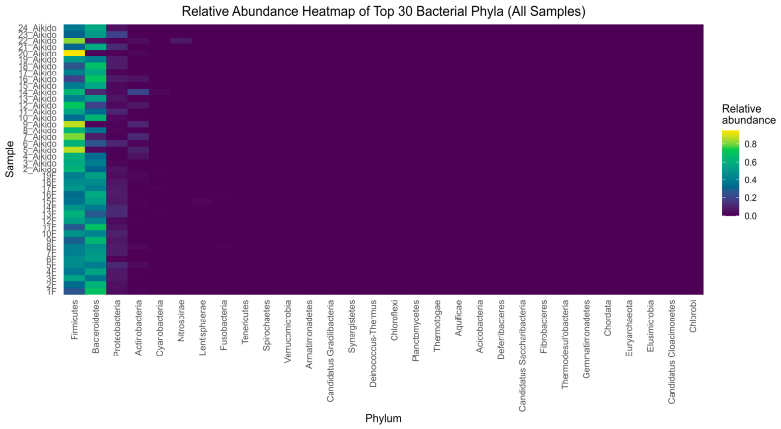


Figure 8. Phylum-level abundance heatmap and significant phyla. Heatmap of Z-score normalized phylum abundances, dominated by Firmicutes and Bacteroidetes.

These results are consistent with the visual patterns observed in the heatmaps at both genus and phylum levels, indicating that Aikido and Tai Chi practitioners exhibit distinct gut microbial compositions across taxonomic tiers. To further explore the taxonomic differences underlying the observed beta-diversity patterns, a heatmap was generated displaying the 20 most prevalent genera across combined sport type and meditation level groups (Figure 9). Overall, the microbial community composition showed variations associated with both the type of practice and meditation level. *Bacteroides* and *Faecalibacterium* were among the most abundant genera across all groups, in line with previous reports describing their widespread presence in the human gut microbiota. Within Aikido practitioners, differences in relative abundance of *Faecalibacterium*, *Roseburia*, *Ruminococcus*, and *Butyricimonas* were descriptively observed across meditation levels. As *Faecalibacterium* and *Roseburia* have been previously associated with short-chain fatty acid production, these taxonomic patterns may warrant further functional investigation; however, no functional or metabolomic measurements were performed in the present study. Conversely, *Alistipes* and *Sutterella* displayed lower relative abundances in higher meditation-level Aikido groups. In Tai Chi practitioners, similar trends were observed, with higher meditation levels showing increased relative abundances of *Faecalibacterium* and *Roseburia*, alongside differences in other taxa when compared with Aikido. For example, *Odoribacter* and *Parabacteroides* were more abundant in higher meditation-level Tai Chi groups than in the highest meditation-level Aikido group. Taken together, these findings suggest that both practice type and meditation level are associated with distinct taxonomic profiles of the gut microbiota. These observations are consistent with beta-diversity patterns observed in PCoA and ANOSIM analyses and should be interpreted as descriptive and hypothesis-generating rather than indicative of specific functional effects. Given the extreme imbalance of meditation-level strata within sport groups, including single-participant cells, interaction analyses should be interpreted exclusively as exploratory signals and not as evidence of differential effects.

3.6. Phylogenetic Cladogram of Differentially Abundant Taxa Across Aikido and Tai Chi

A differential abundance analysis was conducted to assess the gut microbiota composition between participants of Aikido and Tai Chi. The circular cladogram depicts the phylogenetic relationships of the fifty most abundant genera. The colors of the nodes indicate group-specific enrichment; green nodes signify genera that are enriched in the Aikido group, while orange nodes represent those enriched in the Tai Chi group (Figure 10). Genera found to be enriched in Aikido included members of the *Firmicutes* and *Bacteroidetes* phyla, such as *Faecalibacterium*, *Ruminococcus*, *Roseburia*, *Bacteroides*, and *Barnesiella*. In

contrast, genera enriched in Tai Chi included *Prevotella*, *Collinsella*, *Lactobacillus*, *Dialister*, and *Phascolarctobacterium.*

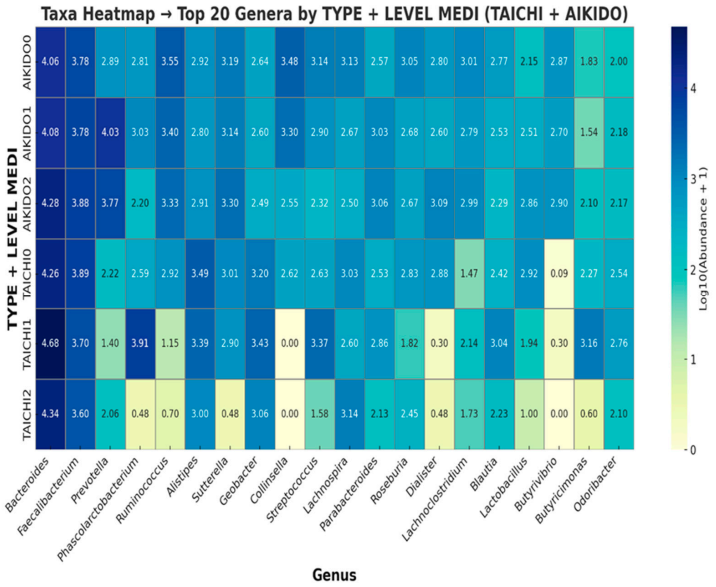


Figure 9. Heatmap of top 20 abundant genera by sport and meditation level. Visualization of community shifts with higher *Faecalibacterium*, *Roseburia*, and *Butyricimonas* in Aikido at higher meditation, and *Odoribacter* and Parabacteroides in Tai Chi at higher meditation.

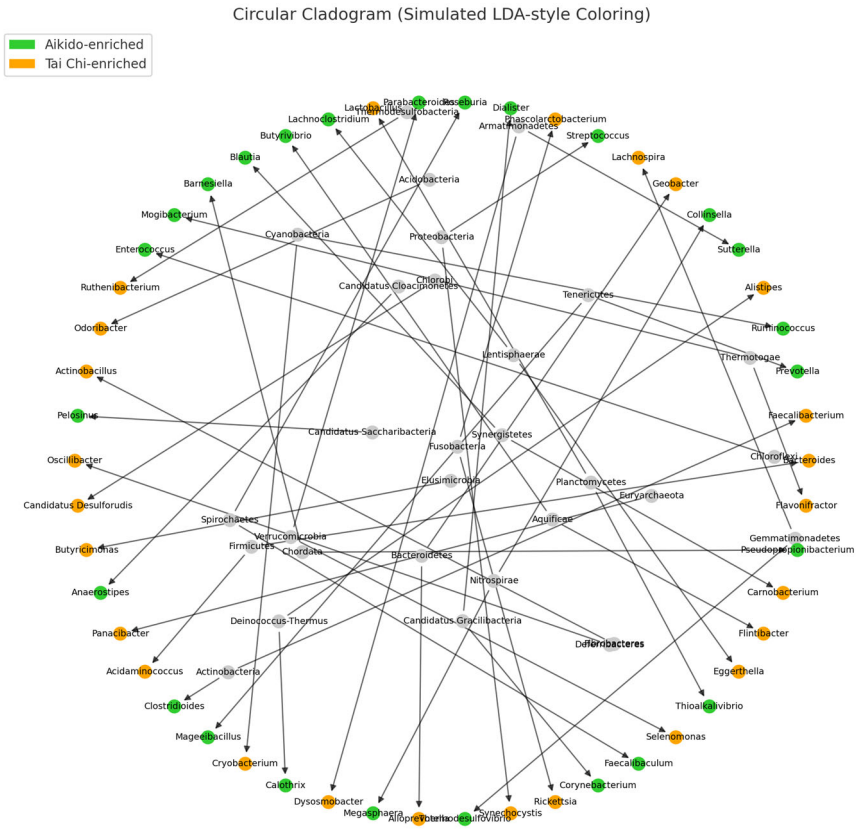


Figure 10. Phylogenetic cladogram of top 50 genera. Circular cladograms show phylogenetic relationships, with green nodes indicating genera enriched in Aikido (e.g., *Faecalibacterium, Roseburia*) and orange nodes indicating genera enriched in Tai Chi (e.g., *Prevotella*, *Collinsella*).

The bar plot depicts the top twenty genera with the greatest log2 fold change between the two groups: genera such as *Faecalibacterium* and *Roseburia* showed higher abundance

in Aikido, while *Prevotella* and *Collinsella* were more abundant in Tai Chi. These findings indicate that participants of Aikido and Tai Chi exhibit distinct profiles in gut microbiota, highlighting significant variations in the abundance of several key genera. Such differences may reflect variations in lifestyle, dietary patterns, or physiological impacts related to these two movement disciplines (Figure 11).

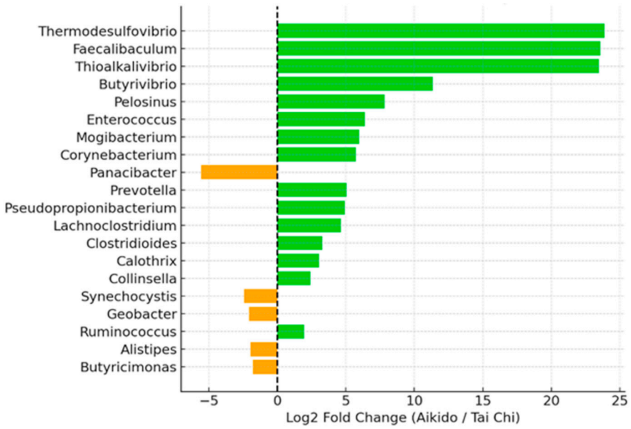


Figure 11. Differential abundance bar plot. Bar plot of the top 20 genera showing the largest log2 fold changes between groups, with *Faecalibacterium* and *Roseburia* enriched in Aikido and *Prevotella* and *Collinsella* enriched in Tai Chi.

4. Discussion

This study was designed as an exploratory, cross-sectional investigation aimed at characterizing gut microbiota profiles within a specific population of non-competitive meditative martial arts practitioners. To our knowledge, this is among the first studies to systematically examine and compare gut microbiome characteristics in practitioners of different meditative movement styles, while also considering meditation depth and gender as relevant analytical dimensions. Accordingly, the findings should be interpreted as associative and hypothesis-generating rather than causal. Observed differences related to practice type and meditation depth reflect within-cohort variability and do not allow attribution of microbiota changes to Tai Chi, Aikido, or meditation per se. Our results indicate that while overall alpha diversity (Shannon and Simpson indices) was comparable across groups (Figure 2), beta-diversity analyses revealed distinct compositional patterns in gut microbiota between Aikido and Tai Chi practitioners (Figures 3 and 4). These findings are consistent with previous evidence suggesting that the qualitative characteristics of physical activity—beyond intensity or duration—may influence gut microbial community structure [5,57,64–67]. Recent evidence supports the notion that differences in exercise modality and movement quality are associated with gut microbiota variation through pathways related to immune activation, gut permeability, and microbial metabolism [5,67,68].

Surprisingly, although Tai Chi is typically seen as a gentler, lower-intensity practice compared to Aikido [30–33] our findings reveal that both disciplines cultivate unique gut microbiota profiles. This is in line with recent research suggesting that elements such as movement style, breath control, and mind–body synchronization have distinct effects on the gut microbiome, irrespective of basic exercise volume [69,70]. The impact of meditation depth on gut microbiota composition was examined through PCoA clustering and Spearman correlation analysis (Figure 5). Although minor clustering by meditation level was observed, no significant correlation was identified between MEDI score and key bacterial genera. This is consistent with growing evidence that, while mindfulness and meditation can affect gut–brain axis function and stress-related physiology [6,13], their direct influence on gut microbiota composition may necessitate longer or more intensive

interventions to become evident [66–68]. It is conceivable that meditation has a more substantial impact on microbial function (e.g., metabolite synthesis) rather than just on taxonomic composition.

Our investigation suggests that both meditation depth and the specific type of meditative movement practice (Tai Chi or Aikido) were associated with differences in gut microbiota composition within this group of practitioners. Beta-diversity patterns, illustrated through Principal Coordinates Analysis (PCoA) based on Bray–Curtis distances, revealed compositional variation of gut microbial communities across meditation levels and between the two practices. The first two PCoA axes accounted for 56.85% and 15.78% of the overall variance, respectively. While the type of sport alone did not show a significant effect on beta diversity, the level of meditation was significantly influential (ANOSIM R = 0.191, $p = 0.035$). An exploratory interaction signal between sport type and meditation level was also observed (ANOSIM R = 0.296, $p = 0.001$). However, given the small and imbalanced size of certain meditation-level subgroups, this finding should be interpreted with caution and considered hypothesis-generating rather than confirmatory. In analyzing PCoA by meditation level within each sport, a clear differentiation of communities was observed, particularly within the Tai Chi group (Figure 5B). Here, individuals who did not meditate (level 0) formed a distinct cluster, whereas those with higher levels of meditation (levels 1 and 2) were more scattered across the first two principal coordinates. Significant taxa contributing to this differentiation included *Sporosarcina*, *Odoribacter*, and *Bacteroides* (Figure 6). In contrast, Aikido practitioners exhibited a more gradual transition in community structure as meditation levels increased (Figure 5A), with genera such as *Bacteroides*, *Parabacteroides*, *Flavonifractor,* and *Pelosinus* influencing the observed variation. At the genus level, a heatmap of mean relative abundance revealed distinct microbial signatures between Aikido and Tai Chi practitioners (Figure 7). Among the 503 genera identified, five showed statistically significant differences between groups after FDR correction (FDR < 0.05): *Thioalkalivibrio*, *Pediococcus*, *Faecalibaculum*, *Butyrivibrio*, and *Rhodanobacter*. Additional genera, including *Bacteroides*, *Faecalibacterium*, *Prevotella*, *Ruminococcus*, and *Alistipes*, displayed group-specific distribution patterns [67,68]. SCFA-associated taxa such as *Faecalibacterium* and *Roseburia* were relatively more abundant in Aikido practitioners with higher meditation levels. Although these taxa have been linked in previous studies to gut barrier integrity and immune modulation, no functional, metagenomic, or metabolomic assessments were performed in the present study. Consequently, any inferred links to SCFA production or gut–brain axis pathways remain speculative and are proposed solely as hypotheses for future investigation [71–73].

In contrast, Tai Chi practitioners exhibited relatively higher abundances of *Prevotella* and *Collinsella*. At the phylum level, five phyla differed between groups after false discovery rate correction (FDR < 0.05): Armatimonadetes, Candidatus Gracilibacteria, Planctomycetes, Nitrospirae, and Fusobacteria (Figure 8). Overall, Firmicutes and Bacteroidetes predominated in both groups, although their relative abundances varied according to practice type and meditation level. These less-characterized phyla have been previously linked to host–microbe interactions, redox metabolism, and immune-related processes [73–75].

Phylogenetic representation of the top 50 genera further illustrated compositional differences between practices (Figure 10). Genera relatively enriched in Aikido included *Faecalibacterium*, *Ruminococcus*, *Roseburia*, *Bacteroides*, and *Barnesiella*, whereas Tai Chi practitioners showed enrichment of *Prevotella*, *Collinsella*, *Lactobacillus*, and *Dialister*. Consistently, bar plots of the top 20 differentially abundant genera highlighted practice-specific distribution patterns (Figure 11).

SCFA-associated genera such as *Faecalibacterium* and *Roseburia* were relatively more abundant in Aikido practitioners with higher meditation levels (Figure 9). Similarly,

Tai Chi practitioners at higher meditation levels displayed enrichment of *Odoribacter* and *Parabacteroides*, taxa previously associated with metabolic and immune-related pathways [57,76–78]. However, no functional, metagenomic, or metabolomic measurements were conducted in the present study, and therefore these taxonomic patterns cannot be directly linked to SCFA production or gut–brain axis signaling.

Taken together, these findings indicate that gut microbiota composition differed according to meditative movement practice and meditation depth within this cohort. These differences were primarily reflected in beta-diversity patterns and taxonomic distributions, while alpha diversity remained largely comparable across groups. Given the observational design, single time-point sampling, and small and imbalanced subgroups, the present results should be interpreted as descriptive and hypothesis-generating.

Several limitations of the present study should be acknowledged. First, the cross-sectional and observational design precludes any inference of causality between meditative movement practices, meditation depth, and gut microbiota composition. Second, the absence of a sedentary control group or practitioners of non-mind–body physical activities limits the ability to attribute observed microbiota differences specifically to Tai Chi, Aikido, or meditation-related components. The overall sample size was modest, and certain subgroup analyses—particularly those stratified by meditation level within each practice—were characterized by small and imbalanced group sizes. As a result, interaction effects should be interpreted with caution and considered exploratory and hypothesis-generating rather than confirmatory. In addition, gut microbiota profiling was based on a single fecal sample per participant, which does not account for intra-individual temporal variability. The use of a 16S rRNA OTU-based analytical pipeline and the lack of functional, metagenomic, metabolomic, or clinical measurements further limit mechanistic interpretation of the observed taxonomic patterns. Consequently, potential links to short-chain fatty acid production, immune modulation, or gut–brain axis signaling cannot be directly assessed. Finally, although several lifestyle and demographic variables were collected, residual confounding cannot be excluded. Taken together, these limitations highlight the exploratory nature of the present work and underscore the need for future longitudinal, adequately powered studies incorporating functional microbiome analyses and clinical or psychological outcomes.

Future longitudinal studies incorporating functional microbiome profiling, metabolomics, and clinical or psychological outcomes will be necessary to clarify the biological relevance and potential mechanisms underlying the observed associations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microorganisms14020275/s1; Table S1: ANOSIM results for gut microbiota composition.

Author Contributions: Conceptualization, T.G., F.V. and V.R.S.; methodology and design, T.G. and F.V.; software and epidemiological analysis, T.G.; validation, T.G., F.V., V.V. and V.R.S.; formal analysis and investigation, T.G. and F.U.; resources, V.R.S.; data curation, T.G., V.V. and F.U.; writing—original draft preparation, T.G. and F.V.; writing—review and editing, T.G., F.V. and V.R.S.; visualization, F.V.; supervision, V.R.S.; project administration, F.V. and V.R.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was carried out respecting the principles of the Declaration of Helsinki. The protocol of the study was approved by the Ethical Board of the University of Rome "Foro Italico" (CAR approval number 204/2024).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data can be obtained from the corresponding author upon reasonable request. All sequencing data generated within the project have been deposited in the Sequence Read Archive (SRA) under BioProject accession number PRJNA1311479.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

WHO	World Health Organization
SCFA	Short-Chain Fatty Acid
BMI	Body Mass Index
MET	Metabolic Equivalent of Task
MEDEQ	Meditation Depth Questionnaire
MEDI	Meditation Depth Index
OTU	Operational Taxonomic Unit
PCR	Polymerase Chain Reaction
PCoA	Principal Coordinates Analysis
ANOSIM	Analysis of Similarities
PA	Physical Activity
FDR	False Discovery Rate
SPSS	Statistical Package for the Social Sciences
IPAQ	International Physical Activity Questionnaire
DNA	Deoxyribonucleic Acid
RDP	Ribosomal Database Project
SD	Standard Deviation

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