The effect of dietary fish oil replacement by microalgae on the gilthead sea bream midgut bacterial microbiota

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ABSTRACT

The aim of this work was to evaluate the effects of dietary microalgae blends as fish oil replacers on the midgut bacterial microbiota of gilthead sea bream (*Sparus aurata*). The control diet (FO) contained only fish oil as source of lipids, EPA and DHA fatty acids, while three experimental diets were used where fish oil was replaced at 67% by one of the following microalgae biomass blends: *Michrochloropsis gaditana* and *Isochrysis* sp. (*Tisochrysis lutea*) (MI), *Phaeodactylum tricornutum* and *Isochrysis* sp. (PI) and *Schizochytrium* sp. and *P. tricornutum* (SP). The midgut bacterial community composition and the dominant OTUs indicated that the sea bream midgut bacterial communities were altered compared to the control diet. Additional evidence from the presumptive bacterial functional pathways suggests that the microalgae-based diets resulted in one overexpressed and one underexpressed pathway. The overexpressed pathway was related to the metabolism of fucose, a major carbohydrate of these microalgae species. This suggests that a new gut microbiota profile was selected due to the microalgae inclusion in the provided diet. All these data combined with the absence of mortality in fish, shows that the gilthead sea bream gut microbiome can smoothly adapt its function according to the metabolic capacity of the microalgae combinations that were used. The MI feed seems to promote several beneficial bacteria with potential probiotic abilities in the fish gut.

Keywords: *Sparus aurata*, fish oil, aquaculture, microbiome, aquafeed

INTRODUCTION

The main sources of proteins and lipids for carnivorous farmed fish, such as the gilthead sea bream (*Sparus aurata)*, are fish meals and fish oils, respectively, which are added in the aquafeeds in order to satisfy fish nutritional requirements and to provide high nutritional quality to the final product (Siddik et al. 2023). Fish meals and fish oils originate mainly from wild small pelagic fish species, making the aquaculture sector dependent on wild fish stocks and reducing, thus, their sustainability (Peron, Mittaine & Le Gallic 2010). For this, several studies have focused on alternative sources of proteins and lipids to reduce the incorporation of fish meals and fish oils in aquafeeds (Oliva-Telles, Enes & Peres 2015, Naylor et al. 2021). Microalgae are suitable and sustainable alternative sources with studies so far showing promising results (Shah et al. 2017, Ahmad, Hassan & Banat 2022).

In fish, the composition of the gut microbiome is mainly determined by host selection (Roeselers et al. 2011) and diet (Liu et al. 2016), while environmental and other stochastic factors seem to be more important for wild fish (Kim et al. 2021, Kormas et al. 2023). The gut microbiome and its interactions with the host influence several important factors for fish health such as nutrition and metabolism (Liu et al. 2021), in maintaining the development and maturation of the immune system (Koch et al. 2018), the nervous system functioning and fish development (Phelps et al. 2017). Furthermore, commensal bacteria have the potential to protect the fish against pathogens (Kelly & Salinas 2017) and when a disease occurs there is a loss of gut microbiota diversity (Felix et al. 2022). For these reasons it is very important to investigate the changes that may occur in the composition of the gut microbiota with changes in the fish's diet.

In this research the following microalgae and oomycete species were used in gilthead sea bream aquafeeds: *Phaeodactylum tricornutum, Tisochrysis lutea* (here referred to as *Isochrysis* sp. due to its reclassification (Bendif et al. 2013)), *Schizochytrium* sp. and *Michrochloropsis gaditana* (formerly *Nannochloropsis gaditana*). *M. gaditana* (Fawley, Jameson & Fawley 2015) is a marine microalga of the class Eustigmatophyceae with high content of eicosapentaenoic acid (EPA) (usually 36% of total fatty acids) (Zanella & Vianello 2020). *P. tricornutum* is a marine diatom of the class Bacillariophyceae, rich in EPA (29% of total fatty acids) (Matos et al. 2016). The oomycete genus *Schizochytrium* belongs to the Thraustochytriaceae family. Members of this genus are especially rich in docosahexaenoic acid (DHA) (41% of total fatty acids) (Karapanagiotidis et al. 2022). *Isochrysis* is a genus of

marine unicellular microalgae that also produces high levels of DHA (49% of total fatty acids) (Ren et al. 2010).

Existing research has evaluated the use of *P. tricornutum* and *M. gaditana* in alternative feeds for gilthead seabream. In particular, *P. tricornutum* when used in experimental feeds, either alone or in combination with *Tetraselmis chuii* and *Bacillus subtilis*, has been linked with reduced gut microbial diversity in gilthead sea bream (Cerezuela et. al 2012). However, the above combination along with dietary inulin and the utilization of its b-glycans, has demonstrated immunostimulant effects, influencing intestinal gene expression (Cerezuela et al. 2013; Reis et al. 2021; Teixeira et al. 2022). The use of *M. gaditana* showed that gut microbiota composition and intestinal integrity remained unaltered (Cerezo-Ortega et al. 2021) or an increase in intestinal microbiota richness without affecting intestinal morphology and function (Jorge et al. 2019). When used in combination with other species (*Chlorella* sp., *Arthrospira* sp., *Tisochrysis lutea*, *Scenedesmus almeriensis*) it was shown to favour the presence of probiotic bacteria in the gut and reduce the presence of pathogenic species (Garcia-Márquez et al. 2023; Garcia-Márquez et al. 2023). To the best of our knowledge there are no studies about the effects of *Isochrysis* sp. and *Schizochytrium* sp. in gilthead sea bream gut microbiota.

These microalgae species when combined in fish diets, appear suitable for replacing the levels of EPA and DHA typically provided by fish oils. However, there are no studies evaluating the potential differences on the midgut microbial composition and function of gilthead seabream when fed with a diet containing these microalgae species in combination. Thus, the aim of this study was to investigate the potential effect on the midgut microbial composition and function of gilthead seabream when fed a diet containing these microalgae species in combination. It is hypothesised that a microalgal-based diets will promote gut microbiota related to the metabolism of these microalgae.

MATERIALS AND METHODS

Sampling. For the needs of the present study, 40 *Sparus aurata* specimens weighing 28-37g were collected from a feeding trial (Gkalogianni et al. 2023) conducted at the experimental facilities of the Department of Agriculture, Ichthyology and Aquatic Environment in 2022. The fish were divided into 4 nutritional groups with each group fed with a different diet for a period of 80 days. Fish of the control group were fed a diet

containing fish oil (FO) as the sole source of EPA and DHA, and fishmeal as a source of marine animal protein. The remaining groups were fed experimental diets, in which 67% of the fish oil used in the control diet was substituted by a mixture of microalgae species, serving as a source of EPA and DHA. Specifically, the PI feed contained a microalgae mixture of *P. tricornutum* and *Isochrysis sp*., the SP a mixture of *Schizochytrium sp.* and *P. tricornutum*, and the MI a mixture of *M. gaditana* and *Isochrysis sp.* Since the microalgae used in the experimental diets were in the form of dry biomass (and not extracted oils), their inclusion in the feed contributed to the overall protein content. For this reason, the percentage of inclusion of fishmeal was reduced accordingly. At the end of the dietary trial, 10 individuals were randomly collected from each dietary group, euthanized with a strong dose of the anesthetic phenoxyethanol and placed directly on ice. For the gut microbiota analysis, the whole intestine of its individual was removed in aseptic conditions, with scissors and scalpel after sterilization with 70% ethanol before each dissection and the midgut part was stored at -80 °C until the DNA extraction procedure.

DNA extraction and sequencing. The DNA of seven gut samples of each feeding group (FO, PI, SP, MI) was extracted using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. Bacterial communities were analysed by 16S rRNA amplicon sequencing targeting the V3-V4 hypervariable region with the primer pair S-D-Bact-0341-b-S-17 and S-D-Bact-0785-a-A-21 (Klindworth et al. 2013). PCR amplification and sequencing was performed at MRDNA Ltd.1 (Shallowater, TX, United States) facilities on a MiSeq using paired end reads (2 × 300 bp) following the manufacturer’s guidelines.

Data analysis. The 16S rRNA sequencing raw data were processed using the MOTHUR MiSeq SOP procedure (Schloss et al. 2013). Raw sequence reads have been deposited in the Short Read Archive () with PRJNA1068122 BioProject accession number. The operational taxonomic units (OTUs) at 97% cutoff similarity level were classified with the SILVA database release 138.1 (Quast et al. 2013, Yilmaz et al. 2014). Identification of the closest relatives of the most abundant OTUs was performed using a Nucleotide Blast (http://blast.ncbi.nlm.nih.gov). The statistical analysis and graphic illustrations were performed using PAleontological STudies (PAST) software (Hammer, Harper & Ryan 2001).

Presumptive functional analysis of midgut microbiota. The PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved STates) (Douglas et al. 2020) software was used to predict the microbiota functional profile (Langille et al. 2013). The predicted functions were searched in the MetaCyc database (Caspi et al.

2013), to be assigned to each metabolic pathway. Also, the KEGG (Kyoto Encyclopedia of Genes and Genomes) database (Kanehisa & Goto 2000, Kanehisa et al. 2023) was used to identify whether the genera of the most important OTUs have all the enzymes for specific metabolic pathways of interest.

RESULTS

Sequencing data and OTUs. After the quality filtering of the amplicon sequencing of the 16S rRNA gene V3–V4 region, a total of 319.103 reads remained. The number of reads ranged from 1.631 to 44.124 reads per sample. A subsample of 2.859 reads per sample was used for the normalization of the data. As a result, two samples (one from each of following treatments: FO and PI) with only 1.631 and 1.867 reads, respectively, were excluded from the rest of the analyses. The total number of OTUs to which the reads were assigned was 519.

Alpha diversity indices. Taxa_S, Shannon_H, Simpson_1-D and Chao-1 indices were calculated to assess the alpha diversity of the gut microbiota of gilthead seabream in the four groups (Table 1). Compared to the control (FO), Shannon_H and Simpson_1-D values showed a slight increase in PI, while they decreased in MI and SP. Chao-1 index values showed a decrease compared to FO in all three groups, while Taxa_S increased in PI and SP and showed a slight decrease in MI. Despite the existing differences, the Bray-Curtis PERMANOVA (permutational analysis of variance) performed (Table S1) showed no statistically significant differences between the indices values of the three experimental groups with FO. The only statistically significant difference was observed in Taxa_S and Shannon_H between PI and MI.

Beta diversity. Non-metric multidimensional scaling analysis (nMDS) was used to evaluate the beta diversity of the samples of the four feeding groups, based on a Bray-Curtis distance matrix (Figure 1). The PI group is clearly separated from the samples of the FO and SP group. The samples of the FO, MI and SP groups overlapped and there was also an overlap between the samples of the PI and MI groups. Group PI was the only treatment with no overlap with FO. PERMANOVA pairwise test (Table S2) was used to further analyze the differences between dietary groups. Significant differences were observed between the PI and FO and between the PI and SP groups.

The unique OTUs in FO, PI, SP and MI groups (Figure S1a) were 40 (7.7% of all OTUs), 61 (11.8%), 148 (28.5%) and 56 (10.8%) respectively. 11.2% of the OTUs were found in all groups (FO, PI, SP, MI). Furthermore, there were 91 unique OTUs in FO compared to PI (30%), 95 unique OTUs compared to MI (34.2%) and 76 unique OTUs compared to SP (19.5%) (Figure S1b). Shared OTUs were in a higher proportion between FO and MI (34.5%) with FO and PI being very close (33%) and, lastly, FO and SP (29%). SP had the highest number of unique OTUs (199 OTUs - 51%) compared to FO, with MI and PI having smaller contributions of unique OTUs (87 OTUs - 31.3% and 112 OTUs -37%, respectively).

Midgut bacterial taxonomic composition. Within the three most abundant phyla in all groups were Proteobacteria and Actinobacteriota with different relative abundance rates (Figure S2). In FO and MI the third most abundant phylum was Firmicutes, while in PI and SP the 3rd most abundant phylum was Verrucomicrobiota and Bacteroidota respectively. In FO, MI and SP, despite differences in either the order of abundance of the phyla (FO-MI) or the phyla present (FO-SP), their ratio values were quite close. In contrast, in PI there was a large increase in the relative abundance of Proteobacteria, since Proteobacteria were x2.1 more abundant than Actinobacteriota and x8.1 than Verrucomicrobiota. Actinobacteriota also had a large difference in relative abundance with the third most abundant phylum, Verrucomicrobiota (x3.8). Regarding other phyla, only in MI the Fusobacteriota occurred with a relative abundance of 4.3% and in PI and SP the Firmicutes with relative abundance of 5.9% and 13.9% respectively.

In all feeding groups the Mycobacteriaceae family was most abundant in the FO, MI and SP and second most abundant in PI (Figure 2). In PI the most abundant family was Rhodobacteraceae, which in MI and SP occured in very low abundances. These abundances were due to the most dominant OTUs, which in FO, MI and SP was OTU001 and OTU004 in PI (Table S3). Nucleotide BLAST search assigned OTU001 as *Mycolicibacterium aurum* and OTU004 as *Yoonia litorea*. Compared to FO, in the other feeding groups the family Rhizobiaceae was not present and the family Xanthomonadaceae was only present in MI (<4% relative abundance). The family Vibrionaceae occurred in MI and PI but it was absent in SP and FO. Treatment MI had the highest number of families contributing >4% relative abundance (nine families), while SP had the lowest (five families).

Most important OTUs. In the dominant OTUs (cumulative relative dominance > 80% - Figure 3) 3 OTUs occured in all feeding groups (OTU001, OTU003, OTU012). OTU003 and OTU012 occured in small proportions in PI (1.67% and 2.44%). From the

Nucleotide BLAST search OTU003 closest relative was the *uncultured Staphylococcus sp.* and OTU012 closest relative was *Cutibacterium acnes*. OTU004, which was the most dominant in PI, was among the dominants of FO and MI, but with low relative abundances (2.95% and 2.16% respectively). Within the dominant OTUs there were several unique ones that occurred in each feeding group.

The qualitative differences already observed in phyla and families were also observed at the level of dominant OTUs in each group. To explore this further, we compared key OTUs between the FO and the three experimental groups. This comparison unveiled which OTUs were added in high abundance, were common in both the FO and experimental feeds, or exclusive to the FO (Figure 4, Table S4). 11 common OTUs were highly abundant in FO compared to MI, PI and SP. Furthermore, among OTUs found in high abundance in both FO and MI, PI, SP 2 OTUs (OTU001, OTU003) were observed in all cases. In OTUs added in high abundance in MI, PI and SP compared to FO, most were unique to each experimental feed, with 2 OTUs (OTU006, OTU027) being common in FO-MI and FO-PI.

Presumptive functional pathways. Compared to FO, one metabolic pathway was considerably overexpressed (FUCCAT-PWY) and one considerably underexpressed (PWY-6470) in all three experimental feeding groups (Figure 5, Table S5). According to the MetaCyc database, the FUCCAT-PWY pathway corresponds to L-fucose degradation, and the PWY-6470 pathway corresponds to peptidoglycan biosynthesis. Several common pathways were overexpressed in MI and PI relatively to FO and the highest number of unique pathways were overexpressed in SP. From the KEGG database it was found that the genomes of most of the OTUs in each of the three experimental feeds (see Fig. 4) contained the genes encoding for the enzymes of the metabolic pathway L-Fucose degradation (Table S6).

DISCUSSION

In this study, it was hypothesized that the inclusion of microalgal-based diets in experimental reared gilthead sea bream would induce structural bacterial microbiota changes adapted to these novel feed ingredients. In similar studies with the use of microalgae in the diet of gilthead sea bream there were varying results. Cerezuela et al. (2012) reported that the dietary inclusion of 100g/kg *Tetraselmis chui* or *P. tricornutum* decreased the intestinal microbial richness with a lower Shannon index value than the control. In the current study, the Shannon index was decreased in fish fed the *Schizochytrium* - *P. tricornutum* (SP) diet

but was slightly increased in fish fed the *P. tricornutum – Isochrysis* diet. Nevertheless, these two diets had more OTUs (Taxa_S) than the control (FO). Jorge et al. (2019) using *M. gaditana* for fishmeal replacement reported a higher, but insignificant to the control, intestinal richness and diversity of the species, while Garcia-Marquez et al. (2023) using a microalgae blend of *Tisochrysis lutea* (member of the *Isochrysis* sp**p**. **group**), *M. gaditana* and *Scenedesmus almeriensis* at 5%, 15% and 25% dietary inclusion levels showed that the microalgae blend promoted an increase in bacterial species diversity and a distinct shift in microbiota fingerprinting as inclusion levels increased. In our results the diet containing *M. gaditana* (MI) showed a decrease in the values of all alpha diversity indices, compared to the control (FO), but not statistically significant. Cerezo-Ortega et al. (2021) also reported no statistically significant differences in microbial diversity indices (alpha and beta diversity) in *Sparus aurata* gut after the inclusion of 5% hydrolysed *M. gaditana*.

Studies with dietary inclusion of *Schizochytrium* sp. in Nile tilapia, zebrafish and rainbow trout (Souza et al. 2020, Shi et al. 2021, Lyons et al. 2016) also showed varied results. Specifically, in Nile tilapia a diet with 1.2% inclusion of *Schizochytrium sp.* led to higher values of the Chao richness index compared to the control group. The authors, reported also higher Inverse Simpson and Shannon values in the *Schizochytrium*-fed fish, but these were not significantly different to those in the control group. In zebrafish, the dietary inclusion at 0, 60 and 120g/kg of *Schizochytrium sp.* resulted in insignificant differences in Chao and Shannon indices among the three groups, but in the beta diversity nMDS showed that both the *Schizochytrium sp.* supplemented diets were separated from the control group (Shi et al. 2021). The diet containing Schizochytrium sp. in our study (SP) had lower values in Shannon-H, Simpson_1-D and Chao-1 indices than the control (FO), but these were not statistically significant and also there were no statistically significant differences in beta diversity compared to the control. Furthermore, a 5% inclusion of *Schizochytrium limacinum* for fish oil replacement in the diet of rainbow trout resulted in a greater level of microbial diversity (Lyons et al. 2016; a fact that was also observed in our results using the SP diet that had a higher OTUs (Taxa_S) index number than the control (FO).

Overall, the effect on diversity is certainly determined by the different species of microalgae and fish and the levels of inclusion of each microalga in the diet. The literature as discussed above in combination with the results in our study shows the various changes that occur as the gut microbiome adapts to the different components added by microalgae to the diet. However, differences in diversity alone cannot show the overall effects and investigation

of changes at the level of bacterial phyla, families, and specific OTUs that modulate the diversity is required.

Most important OTUs. The dominant phyla found (Proteobacteria, Actinobacteriota, Bacteroidota) are common in the gilthead seabream gut microbiome (Kormas et al. 2014, Nikouli et al. 2018, 2019, Cerezo-Ortega et al 2021) and also in other fish species gut microbiome (Llewellyn et al. 2016, Rosenau et al. 2021, Zhang et al. 2022). In PI, however, there was an increased occurrence of Verrucomicrobiota. The Mycobacteriaceae family is the one that is highly abundant in all groups, due to OTU001, which belongs to the genus *Mycolicibacterium*. From the available literature it seems that this genus is not common in the gilthead sea bream gut. It includes some species of pathogenic micro-organisms for humans (*Mycobacterium tuberculosis, Mycobacterium leprae*) but also several species that inhabit a diverse range of environments including water bodies, soil, and metalworking fluids (Gupta, Lo & Son 2018). *Mycobacterium* genus has also been found in the gut of freshwater species (Gallet et al. 2022). The closest relative, *M. aurum* has never been reported as a pathogen, but its possible functions in the gut remain unknown. The Rhodobacteraceae family member OTU004 is the dominant in the PI treatment. According to the Nucleotide BLASt search its closest relative is *Yoonia litorea* (Paracocacceae family). *Y. litorea* though has been recently classified in the Roseobacter clade within the Rhodobacteraceae family (Wirth & Whitman 2018). Later, it was proposed the family Roseobacteraceae for members of the Roseobacter clade (Liang et al. 2021). Misclassifications have been a reoccurring problem between the Paracocacceae and Roseobacteraceae families (Zhang et al. 2023), but *Yoonia* sp. isolates are plylogenetically placed in the Roseobacteraceae family (Feng & Xing 2023). Roseobacter-clade species have been isolated from seawater marine sediments and biofilms, and are often associated with phytoplankton, macroalgae and marine animals (Wirth & Whitman 2018). They have also been found in intestine of finfish and they seem to have probiotic effects (Ringø et al. 2022). OTU003 is highly abundant in FO and MI, PI and SP and its closest relative is *Staphylococcus* sp., while OTU012 is highly abundant in FO, MI and PI and its closest relative is *Cutibacterium acnes*. *Staphylococcus* sp. is a common bacterial genus in healthy fish gut microbiomes and some species may have probiotic effects (Zorriehzahra et al.2015, Borah et al. 2016, El-Saadony et al. 2021). *Cutibacterium acnes* has been found in other fish species and it has been suggested to have potential probiotic features (Nikouli et al. 2021). OTU006 and OTU027, that belong to the *Vibrio* genus are in high abundance in MI and PI compared to FO. *Vibrio* is a common bacterial genus found in fish gut with both pathogenic and non-pathogenic species. Some *Vibrio* species can act as

symbionts assisting in the breakdown of dietary components, with strains found to produce amylase, cellulose and chitinase among others (Egerton et al. 2018). From the rest top abundant OTUs, in MI OTU013, OTU031 and OTU041 are closely related to *Pseudoalteromonas sp.*, *Pseudomonas sp.* and *Bacillus sp.* respectively. Several species of these genera are known to have probiotic effects in the fish gut (Mladineo et al. 2016, Zorriehzahra et al. 2015, Salam et al. 2021). OTU030 was abundant in FO and MI and was related to *Rhodopseudomonas* sp., a genus that is also reported to have probiotic effects in the fish gut (Koga et al. 2022). Thus, from the point of structural changes of the bacterial communities, the MI experimental feed seems to promote several beneficial bacterial species in the gut of gilthead seabream.

L-fucose degradation was the only considerably overexpressed bacterial metabolic pathway in the three experimental feeds in comparison to FO. Fucose is a deoxyhexose that is found as a constituent of exopolysaccharides from microalgae (Ahmed et al. 2013, Liu et al. 2019, Wan et al. 2019). It is also commonly found in animal cell glycans, macroalgae, cyanobacteria and microbial exopolysaccharides (Becker & Lowe 2003). Furthermore, the search of the KEGG database confirms that most of the important bacterial genera found in this study, have all five enzymes to carry out the L-fucose degradation. This suggests that the microalgal-based diets induced a clear gut bacterial response, at the functional level, favoring the proliferation of bacteria which benefit from specific microalgal compounds with potential benefits to the host, too. The degradation of fucose is a likely beneficial-to-the-host service. For example, in humans fucose degraders are known to induce the production of the beneficial short-chain fatty acids (Morrison et al. 2016, Høgsgaard et al. 2023, Fusco et al. 2023). The metabolic fate of the fucose degradation and its possible effect on the sea bream's physiology remains to be specifically investigated in the future.

The only pathway that was considerably underexpressed in the three experimental feeds compared to FO is peptidoglycan biosynthesis. The cell wall of some microalgae species contains peptidoglycan (Agboola et al. 2019, Machado et al. 2022), but the species used in our research do not seem to contain (Domozych et al. 2012, Le Costaouëc et al. 2017, Nadzir et al. 2023). Peptidoglycan forms around 90% of the dry weight of Gram-positive bacteria but only 10% of Gram-negative strains (Malanovic & Lohner 2016). The relative abundance of Gram-negative phyla and families like Vibrionaceae in PI and MI and Bacteroidota in SP is higher than FO. Finally, SP was the only treatment with unique overexpressed pathways along with the highest number of unique OTUs compared to FO. however, it seems that the unique OTUs are not responsible for these specific pathways (data

not shown). This microalgal feed inclusion requires further and more focused investigation regarding its impact on the sea bream's gut microbiome.

In conclusion, this study showed that the inclusion of three microalgal-based diets induced both structural and functional changes in the sea bream's gut microbiome. The structural changes in each dietary treatment were considerable compared to the control diet. However, the total midgut bacterial communities which were favoured by the microalgal inclusion are inferred to be able to metabolise L-fucose, a major microalgal carbohydrate.

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Table 1. Alpha diversity indices for each treatment. OTUs: operational taxonomic units.

Dietary treatment	OTUs (Taxa_S)	Shannon H	Simpson 1-D	Chao-1
Fish oil	53 ± 9.4	2.02 ± 0.570	0.75 ± 0.170	152.6 ± 85.38
Phaeodactylum + *Isochrysis*	57 ± 14.2	2.07 ± 0.366	0.77 ± 0.081	115.7 ± 26.74
Schizochytrium + *Phaeodactylum*	64 ± 63.3	1.80 ± 0.984	0.65 ± 0.265	123.2 ± 70.58
Microchloropsis + *Isochrysis*	44 ± 8.0	1.41 ± 0.657	0.56 ± 0.254	104.3 ± 37.40

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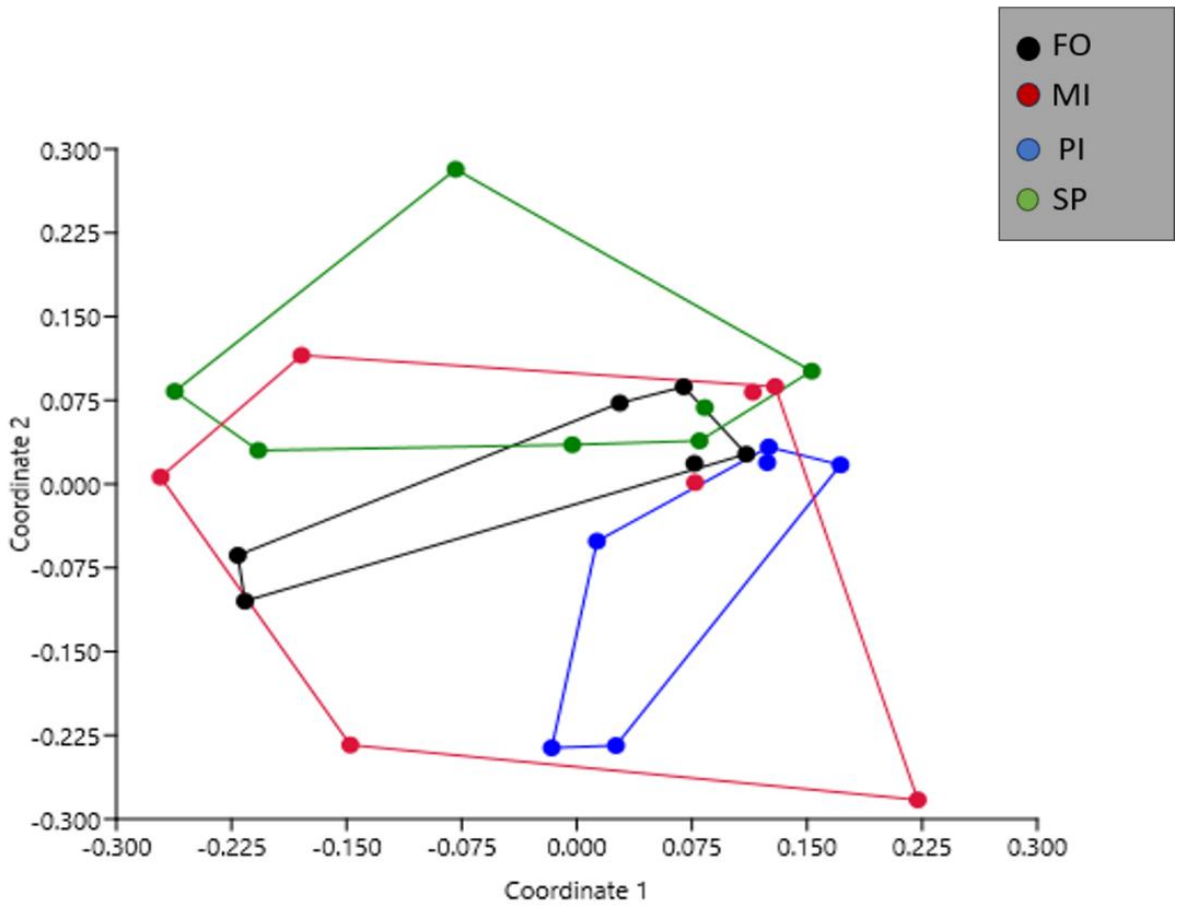

Figure 1. Non-metric multidimensional scaling (nMDS) plot for all the bacterial communities of all samples of the four dietary groups based on Bray-Curtis distances. The MI samples have the largest range, PI does not coincide with FO and SP.

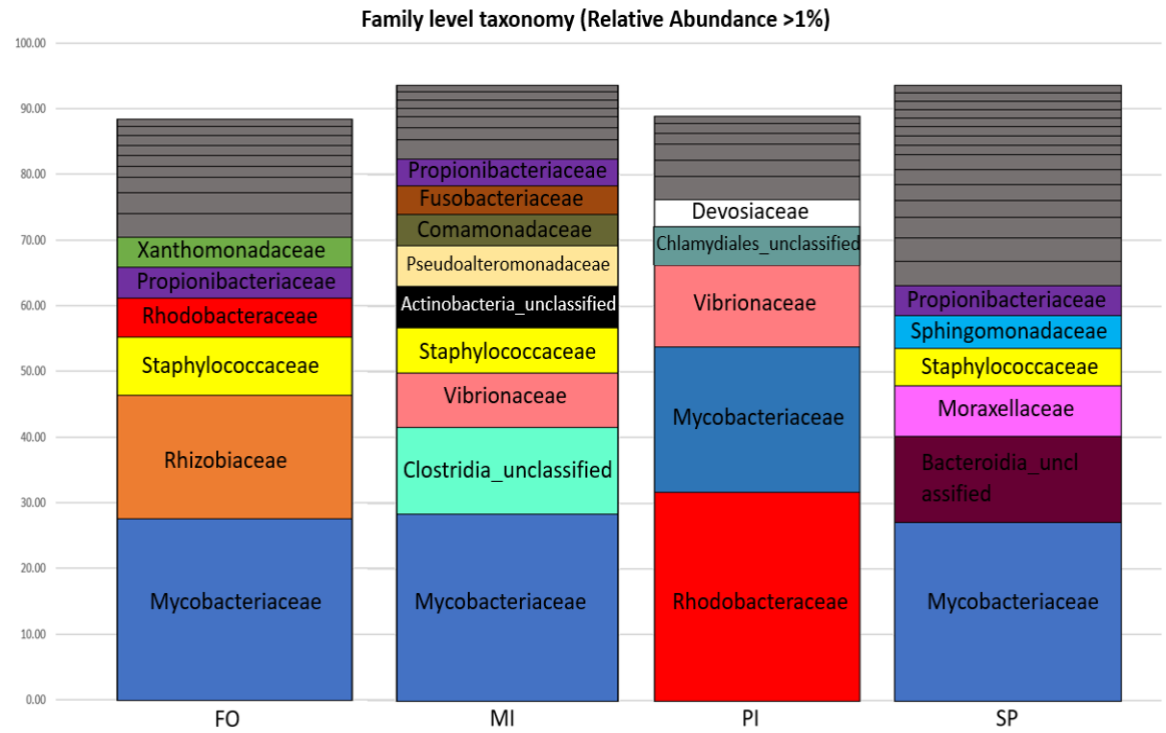

Figure 2. Taxonomic composition of the bacterial families in each dietary group. Relative abundances were calculated adding the relative abundance (calculated based on the average samples reads) of all OTUs belonging to each family. Only the families with relative abundance > 1% were considered for the diagram. Families with relative abundance < 4% are depicted in grey.

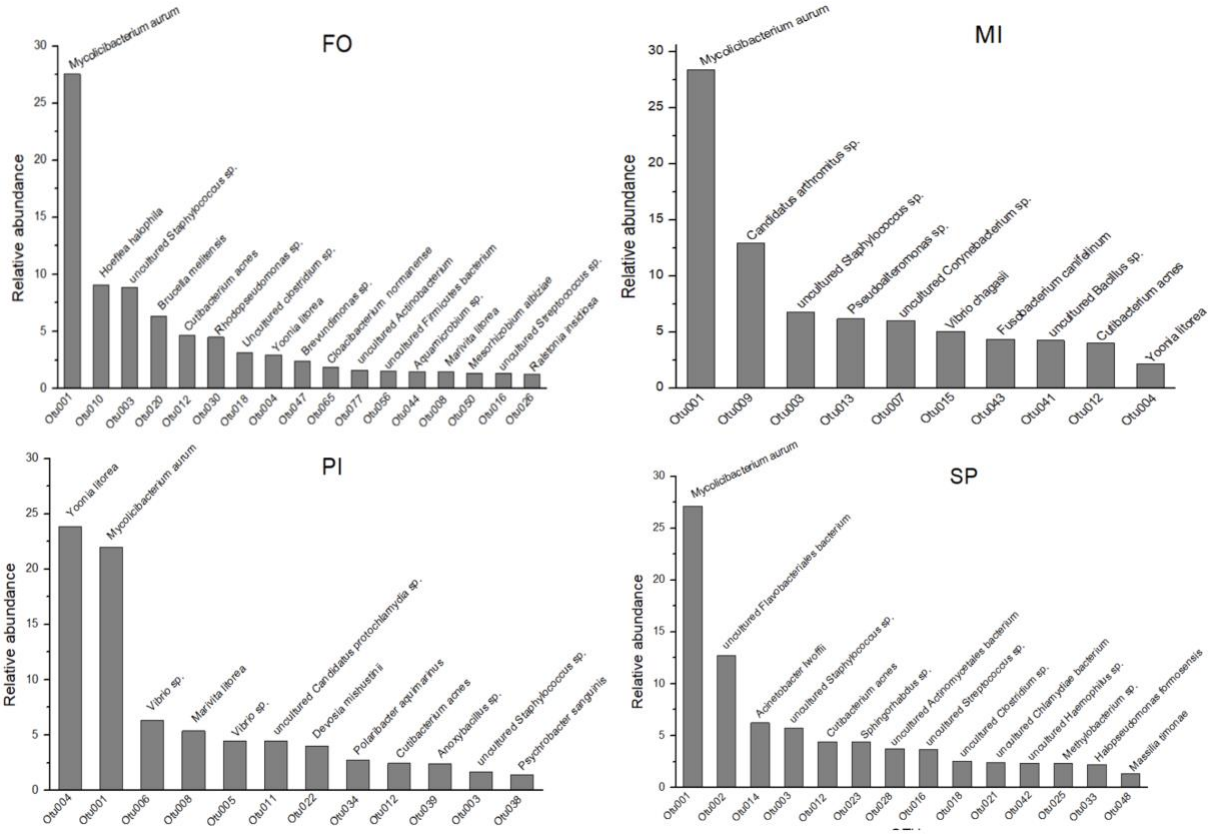

Figure 3. Most dominant OTUs in each dietary group (cumulative relative dominance > 80%) and closest relative (Nucleotide BLAST).

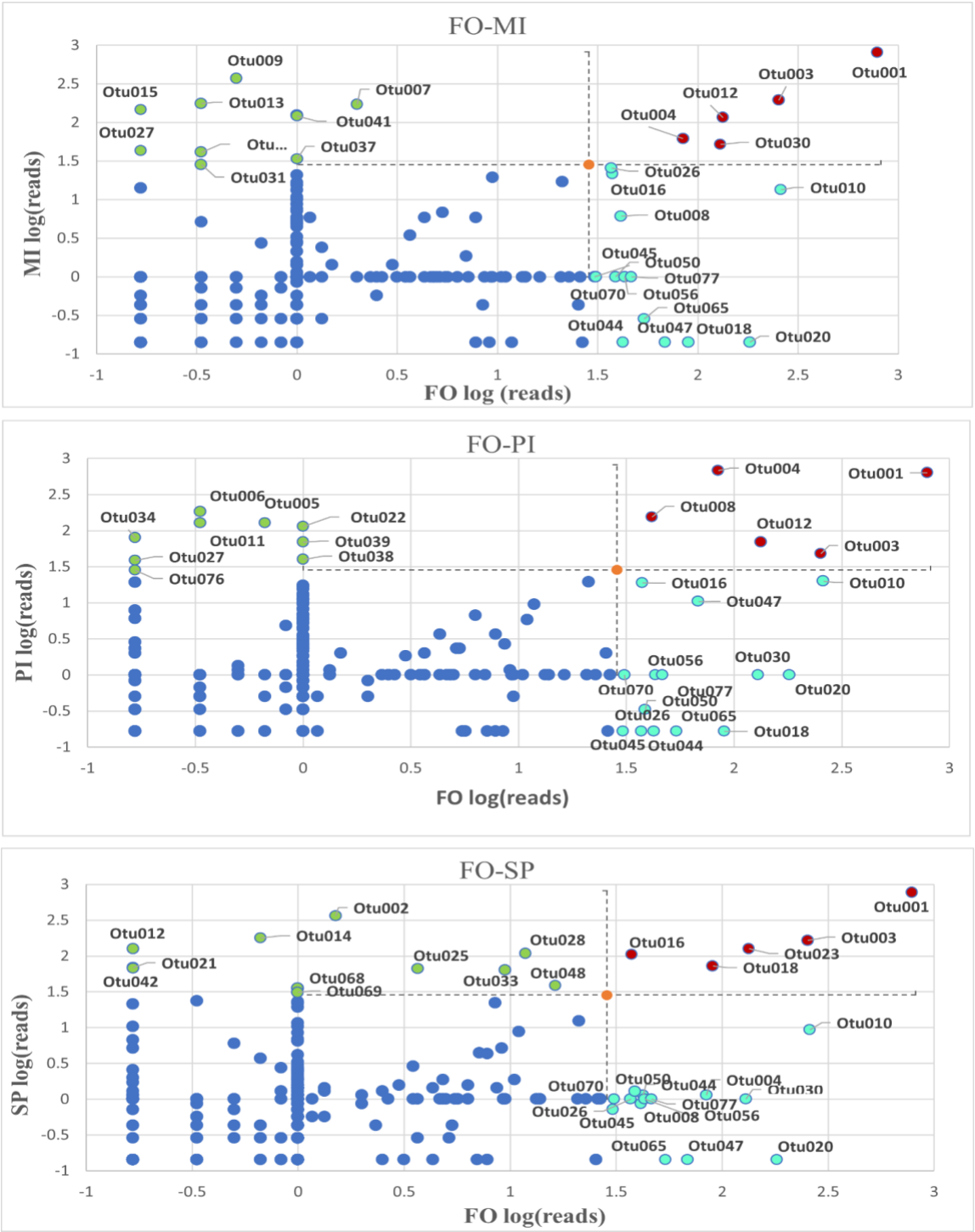

Figure 4. Relative abundance of the FO reads versus their respective values of the PI, MI and SP. OTUs depicted in green are in >1% relative abundance in PI or MI or SP but not in FO, OTUs depicted in red are in high abundance in FO and PI, MI and SP concomitantly and OTUs depicted in pale blue are in high abundance in FO but not in PI, MI and SP. The limit set (orange point) is the log of 1% of the total reads.

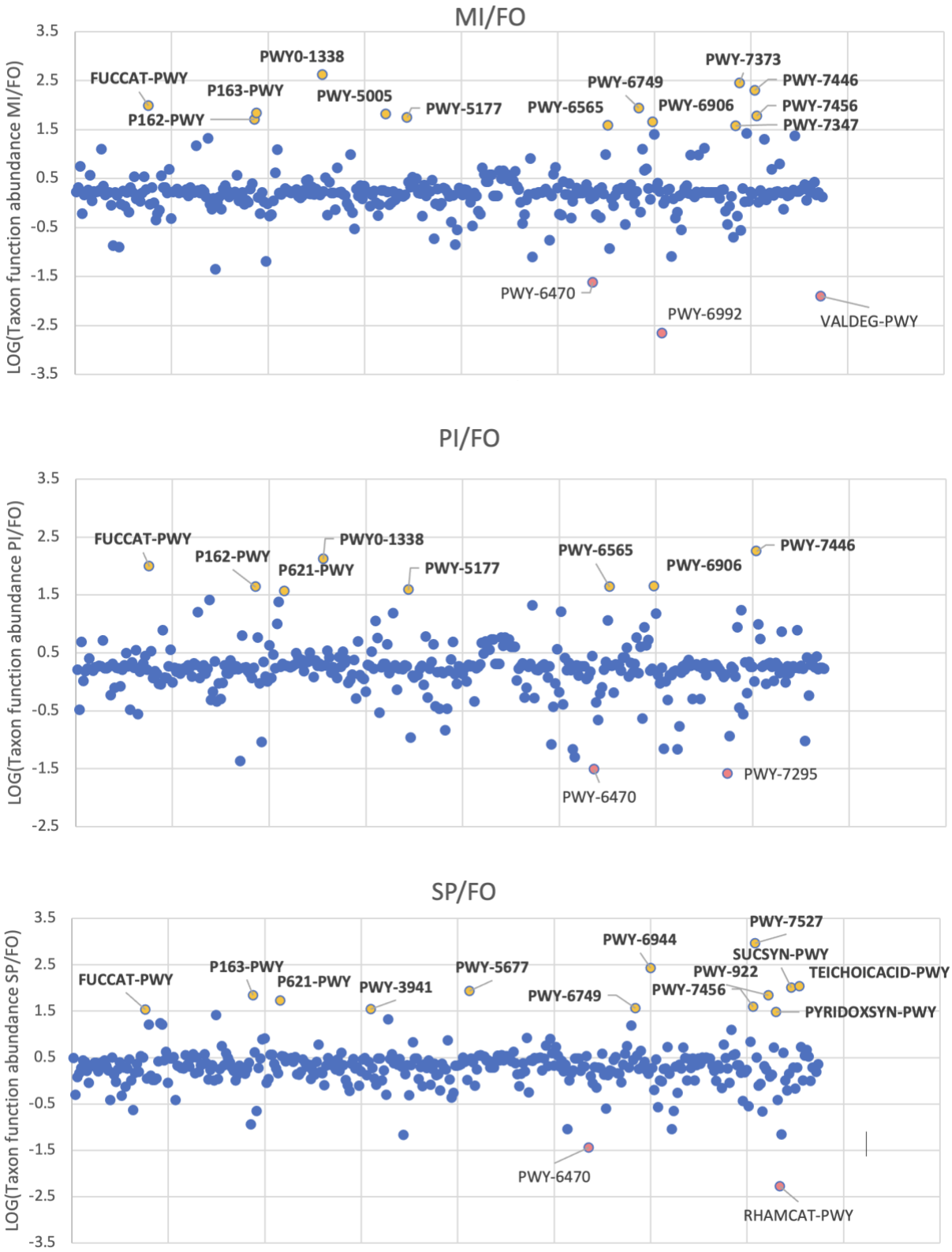

Figure 5. Scatter plots with every pathway in x axis and the log of the ratio PI/FO, MI/FO and SP/FO of the Taxon function abundance in y axis. Pathways > 1.5 are considered overexpressed in PI, MI and SP respectively compared to FO and pathways < -1.5 are considered underexpressed.