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Gracilaria gracilis and *Nannochloropsis oceanica*, singly or in combination, in diets alter the intestinal microbiota of European seabass (*Dicentrarchus labrax*)

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Algae feeds and fish gut microbiota have been given importance in the past few years because of the necessity to rely on sustainable ingredients in aquafeeds and the link of host-associated microbes to organismal health. But little is known about the potential of algae, particularly of micro- and macroalgae combination, to shape the intestinal bacterial communities. Hence, in the present work, the 16S rRNA gene sequencing technique was employed to unravel the effects of the seaweed *Gracilaria gracilis* and the microalga *Nannochloropsis oceanica* - included either singly or in combination in the diets of European seabass - on the diversities and composition of the gut bacterial communities. Results indicated that 8% inclusion of either *G. gracilis* (GRA) or *N. oceanica* (NAN) led to a reduction in the gut microbial diversity. On the other hand, inclusion of the micro- and macroalga in a blend (NANGRA) mitigated these plausible effects on the intestinal bacterial communities. The core microbiota of European seabass was composed of both beneficial (*Lactobacillus* and *Cetobacterium*) and potentially pathogenic (*Flavobacterium*) bacteria. The GRA diet was associated with a lower abundance of carbohydrate degraders and also promoted the growth of bacteria capable of outcompeting fish pathogens (*Sulfitobacter* and *Methylobacterium*). On the other hand, the NAN diet led to a higher representation of the genus *Bacillus*, with probiotic potential, accompanied by a decrease in *Vibrio*, a genus encompassing several fish pathogenic species. These findings demonstrate the ability of micro- and macroalgae to modulate the gut microbiota of European seabass, with plausible implications to host gut homeostasis.

KEYWORDS

seaweed, microalga, algae blend, European seabass, intestinal microbiota

Introduction

Fish intestinal microbiota has become one of the most studied topics in the field of aquaculture, by following the trends in human and mammalian research (Diwan et al., 2022). The complex and dynamic assemblage of microorganisms in the gut microbiota is known to have crucial implications on key physiological functions (Cerf-Bensussan and Gaboriau-Routhiau, 2010; Egerton et al., 2018). Since the intestinal bacterial communities of aquatic animals are vastly shaped by diet, it is expected that nutritional manipulation of fish gut microbiota using added-value products can produce intended benefits on fish welfare and nutrition (Yukgehnaish et al., 2020). In this context, it is worth mentioning that algae are natural products and are rich in biologically active substances capable of promoting fish growth and improving the immune status and disease resistance (Wan et al., 2019; Valente et al., 2021). Specifically, two algae that have been recently explored by the feed industry are the macroalga *Gracilaria gracilis* and the microalga *Nannochloropsis oceanica*. Although there are some reports on the effect of these algae on fish growth performance, immunity, oxidative status and intestine histomorphology (Batista et al., 2020; Passos et al., 2021; Sørensen et al., 2021), little is known about their impact on the intestinal microbiota of farmed fish.

The core research on dietary modulation of fish gut microbiota has been undertaken to reveal the effect of either probiotics and prebiotics or dietary proteins and lipids (Egerton et al., 2018; Pérez-Pascual et al., 2020; Vargas-Albores et al., 2021). Studies that evaluated the changes in intestinal microbiota of farmed fish fed algae-supplemented diets have also emerged more recently (Keating et al., 2021; Sagaram et al., 2021). Other studies have focused on the impact of algae as feed ingredients (replaced fish meal/fish oil) (Rico et al., 2016; Lyons et al., 2017; Li et al., 2022) or feed additives (Jorge et al., 2019; Cerezo et al., 2022). Replacing fish meal (partially) with the macroalgae *G. cornea* or *Ulva rigida* altered the diversity of bacteria in gilthead seabream (*Sparus aurata*) intestine: while 15% substitution of both the seaweeds increased the diversity, 25% substitution of *U. rigida* decreased it (Rico et al., 2016). Li et al. (2022) reported the effects of total fish meal replacement with the microalga *Chlorella vulgaris* on the intestinal microbiota of largemouth bass (*Micropterus salmoides*); the abundance of beneficial taxa (e.g., *Cetobacterium* that are known as vitamin B_{12} producers) was increased without any differences in alpha diversity compared to the fish meal group. On the other hand, in rainbow trout (*Oncorhynchus mykiss*), 5% dietary *Schizochytrium limacinum* as partial fish oil substitute significantly increased the intestinal bacterial diversity without changing the overall microbial community structure. Nevertheless, some microbial groups including members of the lactic acid bacteria, which are commonly considered beneficial, had an increased representation in the *S. limacinum* group (Lyons et al., 2017). Short-term feeding of gilthead seabream with diets supplemented with *N. gaditana* at 7.5 g kg^{-1} led to an increased richness of the gut microbiota, but not significant when compared with fish fed the control diet (Jorge et al., 2019). Similarly, the diversity of gut microbiota was not altered in Atlantic cod (*Gadus morhua*) fed diets supplemented with *U. rigida* (10%) compared to the control group (Keating et al., 2021).

Based on the results reported in the available literature, micro- and macroalgae, either as ingredients or supplements, seem to modulate the gut microbiota of farmed fish, but the effects are species-specific and vastly dependent on the type of the algae and their inclusion levels. Moreover, most studies have reported the effects of individual algae. A mix of micro- and macroalgae in aquafeeds can impart synergistic modulatory effects on the gut microbiota. However, this topic is underexplored and deserves more attention because both micro- and macroalgae can be future feed ingredients/additives. Here we report for the first time the impact of the seaweed *G. gracilis* and the microalga *N. oceanica*, incorporated singly or in combination, on the composition of the microbial communities in the posterior intestine of European seabass (*Dicentrarchus labrax*), which is one of the most important farmed fish species of the Mediterranean region.

Material and methods

Ethical statement

The trial was performed by accredited scientists, and the animal handling and sampling procedures were in compliance with the guidelines of the European Union (directive 2010/63/EU) and Portuguese law (Decreto-Lei no. 113/2013, de 7 de Agosto) on the protection of animals used for scientific purposes. Review of the ethical process concerning all animal handling and sampling procedures was performed by CIIMAR animal welfare body (ORBEA-CIIMAR) and approved by national competent authorities.

Experimental diets, feeding trial, and sampling

Details of the experimental design and diets for the feeding trial, as well as zootechnical data can be found in Batista et al. (2020). Briefly, European seabass juveniles (approximately 6 months of age) from a commercial fish farm (SONRIONANSA S.L., Cantabria, Spain) were transported to CIIMAR facilities in Matosinhos, Portugal. Following a 2-week quarantine, twelve

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homogeneous groups (average body weight of 29.7 ± 0.02 g and total length of 13.7 ± 0.08 cm) of nineteen fish were transferred into 50-L fiberglass tanks of a saltwater recirculation system (RAS, density of 11.3 kg m^{-3}). The RAS conditions were as follows: water temperature of 21 °C, salinity of 35‰, flow rate at 4 L min^{-1} and 12 h light/12 h dark photoperiod regime.

The experimental diets consisted of four isoproteic (53% dry matter, DM) and isolipidic (17% DM) diets supplied by Sparos Lda.: a commercial feed-based diet (CTRL) and three experimental diets with either 8% *G. gracilis* (GRA) or 8% *N. oceanica* (NAN) or a blend of 4% of each alga (NANGRA), included at the expense of fish meal and wheat meal. Ingredients and proximate composition of the experimental diets are summarized in Figure 1. To unravel the impact of the macroalga *G. gracilis* and the microalga *N. oceanica*, added singly or in combination in diets, on European seabass intestinal microbiota, fish were fed the experimental diets for 106 days. Fish in triplicate tanks per dietary treatment were fed three times a day until apparent satiation. The biotic conditions (e.g., temperature and salinity), known to have a greater impact on the gut microbiota, were kept constant throughout the feeding trial.

At the end of the 15-week feeding trial, fish were starved for 24 h and sacrificed by a sharp blow on the head before sampling the tissues. The gastrointestinal tract of four fish per tank (12 fish per treatment) was removed under sterile conditions and the posterior intestine (section preceding the ileorectal valve) was opened using sterile scissors. A sterile swab (Copan Italia, Italy) was used to collect the mucus from the posterior portion. The swab was then placed in a sterile tube and immediately frozen in liquid nitrogen. Samples were stored at -80 °C until further analysis.

DNA extraction for 16S rRNA sequencing

All the procedures mentioned here were performed under sterile conditions. For DNA extraction, three samples per treatment were randomly selected to divide samples into 4 batches. Genomic DNA was extracted from all samples using QIAamp DNA stool Mini Kit (Qiagen, Germany) according to the manufacturer's protocol, with some modifications. Briefly, swabs with mucus from the posterior intestine were first transferred to 5 mL tubes containing 1.4- and 0.1-mm Zirconium oxide beads (Cayman chemical, USA) and 2 mL of InhibitEX buffer (Qiagen). Afterwards, samples were homogenized using Precellys® Evolution tissue homogenizer at 6,000 RPM (3 cycles of 30 s, with a 30 s pause in-between) following centrifugation for 5 min at 12,000 *g*. The obtained pellet was then resuspended, transferred to new 2 mL tubes, and incubated at 70 °C for 20 min. Following incubation, samples were mixed with a vortex for 1 min and centrifuged for 1 min at 12,000 *g*. The supernatant (600 µL) was transferred into a 2 mL tube with 25 µL of proteinase K, and the following steps of the DNA extraction proceeded according to the manufacturer's protocol (QIAamp DNA Stool Mini Kit). Finally, DNA was eluted using 75 µL of ATE buffer and DNA quantity was checked using Qubit™ dsDNA Assay (Thermo Fisher Scientific Inc., USA).

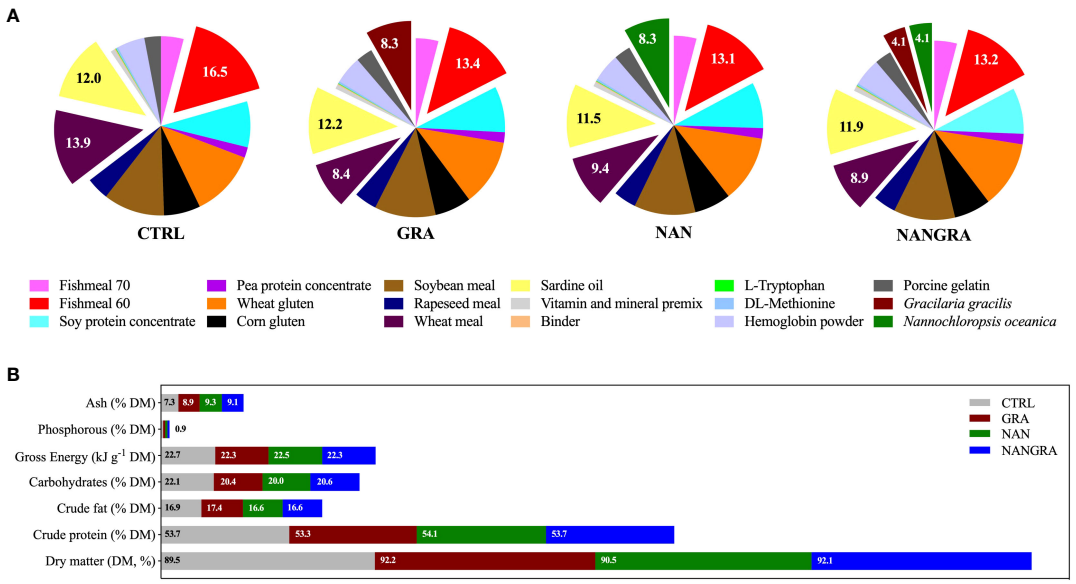

FIGURE 1
Ingredients in % **(A)** and proximate composition in % DM **(B)** of the experimental diets. Adapted from Batista et al. (2020).

Amplicon library preparation and sequencing

Amplicon libraries were prepared under sterile conditions. The first PCR reaction targeted the hypervariable V3-V4 region of the 16S rRNA gene, using specific bacterial primers 341F (5' CCTACGGGNGGCWGCAG 3') and 805R (5' GACTACN VGGGTWTCTAATCC 3') (Klindworth et al., 2013) flanked by Illumina adapters (~ 460 bp; Illumina, USA). The PCR reactions were performed in triplicate for each sample in a 25 µL final volume, with 1 µL of each primer (10 µM), 2.5 µl of DNA template (5 ng/µL), 8 µL of water, and using 12.5 µL of Amplitaq Gold Q5® (Thermo Fisher Scientific Inc.). Thermal cycling conditions were as follows: initial denaturation step at 95°C for 10 min, 37 cycles at 95°C for 30 s, 57°C for 30 s, 72°C for 1 min, and the final extension step at 72°C for 7 min. Negative PCR controls, without DNA template, were also included. At the end of the amplification, the replicates for each sample were pooled together for the subsequent procedures. The amplified products were visualized on an agarose gel (1.5%), and the CleanNGS system (CleanNA, Netherlands) was used to purify the PCR products, according to the manufacturer's recommendations.

A second PCR (index PCR) was performed using the purified products, using Nextera XT Index primers (Illumina) and with the following thermal cycling conditions: 95 °C for 3 min, 8 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 30 min, and a final step at 72 °C for 5 min (16S Metagenomic Sequencing Library Preparation, Illumina). The CleanNGS system (CleanNA) was then used to purify the amplicon libraries. Quality of the obtained libraries was evaluated on a Tapestation 2200 platform (Agilent Technologies, USA), and the libraries were subsequently quantified using the Quant-IT PicoGreen dsDNA assay kit (Thermo Fisher Scientific Inc.) and the Synergy2 microplate reader (Biotek, USA). Thereafter, the pooled library was quantified by Real-Time qPCR LightCycler 480 (Roche, Switzerland), using the KAPA Library quantification kit (Roche). The libraries were sequenced on an Illumina® MiSeq (PE300) platform (MiSeq Control Software 2.5.0.5 and Real-Time Analysis software 1.18.54.0).

Sequence data analysis

Raw sequence data is deposited in the Sequence Read Archive (SRA) and the accession number is PRJNA867546. The obtained reads were first truncated at 270 bp by VSEARCH (Rognes et al., 2016). Next, MICCA pipeline (v1.7.2) (Albanese et al., 2015) was used to further process the reads. Employing MICCA, the sequences were merged at a minimum overlap length of 60 bp and a maximum mismatch of 20 bp. For further analysis, reads without primers were discarded and the forward and reverse primers were trimmed off from the merged reads. Next, sequences with an expected error rate of > 0.75 (Edgar and Flyvbjerg, 2015) were filtered and sequences shorter than 400 bp were removed. The "*de novo* UNOISE" method implemented in MICCA was employed to perform denoising on the filtered reads. The MICCA pipeline uses UNOISE3 algorithm (Edgar, 2016) that corrects sequencing errors and determines true biological sequences at single-nucleotide resolution. Chimera and mitochondrial sequences were removed, and the amplicon sequence variants (ASVs) were generated for the downstream analyses. The RDP classifier was used to assign taxonomies to the bacterial ASVs. The alignment of the sequences was then performed using the NAST multiple sequence aligner (DeSantis et al., 2006), and a phylogenetic tree was generated using the FastTree software within the MICCA pipeline.

Phylogenetic analysis

Blastn (Chen et al., 2015) of the Basic Local Alignment Search Tool (BLAST) was employed to investigate the similarities of the sequences of the identified ASVs and bacterial sequences from the NCBI Reference Sequence Database. Bacterial sequences with 100% match/similarity were selected for the phylogenetic analysis. Thereafter, these sequences were imported into Molecular Evolutionary Genetics Analysis (MEGAX) software (Kumar et al., 2018) and multiple sequence alignment of the ASVs and sequences with high hits in BLASTn was performed using ClustalW aligner (Version 2.0) (Larkin et al., 2007). Phylogenetic analysis of the sequences was performed using the unweighted average binding among clusters (Unweighted Pair Group Method with Arithmetic mean, UPGMA) approach (Rédei (Ed.), 2008), by applying default parameters and 500 bootstrap replicates. The generated phylogenetic trees (using maximum likelihood inference) were visualized using MEGAX software.

Statistical analysis

All statistical analysis were performed on R studio version 1.4.1103. The packages "iNEXT" and "phyloseq" were used to calculate alpha diversity: overall species richness (function "ChaoRichnes"), Shannon diversity (function "ChaoShannon") and Simpson diversity (function "ChaoSimpson"). Faith's phylogenetic diversity was calculated using the function "pd" in package "picante" to reveal the losses and gains in species. Kruskal-Wallis' test followed by Dunn's test were employed to understand the differences between the experimental groups. Then employing the functions in "ggplot2", plots were generated for overall species richness, Shannon diversity, Simpson diversity and Faith's phylogenetic diversity (Hsieh et al., 2016).

For the bacterial beta diversity analysis, weighted UniFrac distances were employed (Lozupone and Knight, 2005), and beta diversity was visualized on a principal coordinates analysis (PCoA) plot. Dispersions of the communities were first analyzed using "betadisper" and significant dissimilarities between the communities were then determined using Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA with 9,999 permutations) (Anderson, 2001), implemented in "adonis" function of the vegan R-package (Oksanen et al., 2013), followed by pairwise comparisons. The package "microbiome" was employed to determine relative abundance of core taxa (Lahti et al., 2017). To identify the differentially abundant ASVs, the non-rarefied data (McMurdie and Holmes, 2014) was analyzed using the "DESeq2" package (Love et al., 2014) as rarefied data is reported to reduce the statistical power of the analysis (Weiss et al., 2017).

Results

Composition of the intestinal microbiota of European seabass fed the algae diets

All samples were sequenced in a single MiSeq run, generating a total of 4,674,383 high-quality reads with an average of 101,617 reads per sample. To account for the read count variation in the different samples, the reads were rarefied to 18,000 sequences per sample; to obtain a uniform sampling depth for comparing the different groups. Out of the 45 samples, two libraries with several reads below the cut off were discarded. A total of 43 samples were used for the downstream analysis – 11 samples for the CTRL group, 11 samples for the GRA group, 12 samples for the NAN group and 9 samples for the NANGRA group. Across all samples, 4371 ASVs were identified after denoising, belonging to 26 phyla and 306 genera.

Concerning the abundances of the intestinal bacteria of European seabass, the 26 bacterial phyla identified across all samples are displayed in Figure 2A (relative abundance per sample) and Supplementary Figure 1A (abundance per dietary treatment). The most abundant phyla were Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria, Nitrospirae and Parcubacteria (Figure 2B). Across all experimental groups, Proteobacteria was the most abundant phylum, with an average relative abundance of 35.25% in the CTRL group, 53.29% in the GRA group, 34.93% in the NAN group and 40.45% in the NANGRA group. The second most abundant phylum for the CTRL group was Bacteroidetes (12.06%), Actinobacteria for the GRA group (11.83%), and Firmicutes for the NAN and NANGRA groups (20.98 and 14.66%, respectively). The third most abundant phyla were Nitrospirae for the CTRL group (10.21%), Bacteroidetes for both GRA and NANGRA groups (9.61 and 11.46%, respectively), and Actinobacteria for the NAN group (12.65%) (Table 1).

The abundance of the dominant genera per dietary treatment are presented in Supplementary Figure 1B. The dominant genera included *Acinetobacter*, *Cetobacterium*, *Corynebacterium*, *Enhydrobacter*, *Flavobacterium*, *Micrococcus*, *Nitrospira*, *Parcubacteria*, *Prevotella*, *Pseudoalteromonas*, *Sphingomonas* and *Streptococcus*, but we did not find a clear dominance of specific genera across all intestinal samples (Figure 2C). The proportions of the dominant genera varied according to dietary treatment: the most abundant genus for the CTRL group was *Nitrospira* (10.03%); for the GRA group the most abundant taxa were *Pseudoalteromonas* (7.57%) and *Acinetobacter* (7.02%); for the NAN group *Streptococcus* had the highest proportion (6.54%); and for the NANGRA group *Acinetobacter* had a higher representation (8.23%) (Table 1).

Impact of the algae diets on the diversity of the intestinal microbiota

The alpha diversity analysis, displayed in Figure 3A, was evaluated using the ecological diversity measures Chao richness (measure of species richness), Shannon diversity (effective number of common species that indicates the even distribution of microbes) and Simpson diversity (effective number of dominant species). The overall species richness was significantly lower in fish that consumed the GRA diet compared to CTRL ($P = 0.003$), but not compared to fish fed the NAN ($P = 0.06$) and NANGRA ($P = 0.36$) diets (Figure 3A1; Kruskal-Wallis' chi-squared = 13.467; $P = 0.0037$). The microbial evenness of the populations (Shannon diversity) in single algae groups (GRA and NAN) was significantly lower than in CTRL-fed fish ($P = 0.009$), but a similar difference was not observed for fish that consumed the algae blend (NANGRA; $P = 0.14$) (Figure 3A2; Kruskal-Wallis' chi-squared = 12.593; $P = 0.0056$). Similarly, fish that consumed both GRA and NAN diets presented a significant reduction in the effective number of dominant species (Simpson diversity) compared to the CTRL fish ($P = 0.02$ and $P = 0.01$, respectively), while such differences were not observed in NANGRA fish ($P = 0.15$) (Figure 3A3; Kruskal-Wallis' chi-squared = 11.442; $P = 0.0096$).

Faith's phylogenetic diversity was also lower in fish fed the GRA ($P = 0.002$) and NAN ($P = 0.04$) diets compared to CTRL, but once again not in fish that consumed the blend diet ($P = 0.29$). Fish fed the GRA diet had also a significantly lower Faith's phylogenetic diversity compared to the NANGRA fish ($P = 0.04$) (Figure 3B; Kruskal-Wallis' chi-squared = 13.323; $P = 0.0025$). For the beta diversity analysis, we incorporated weighted UniFrac distance metric to understand the dissimilarities of

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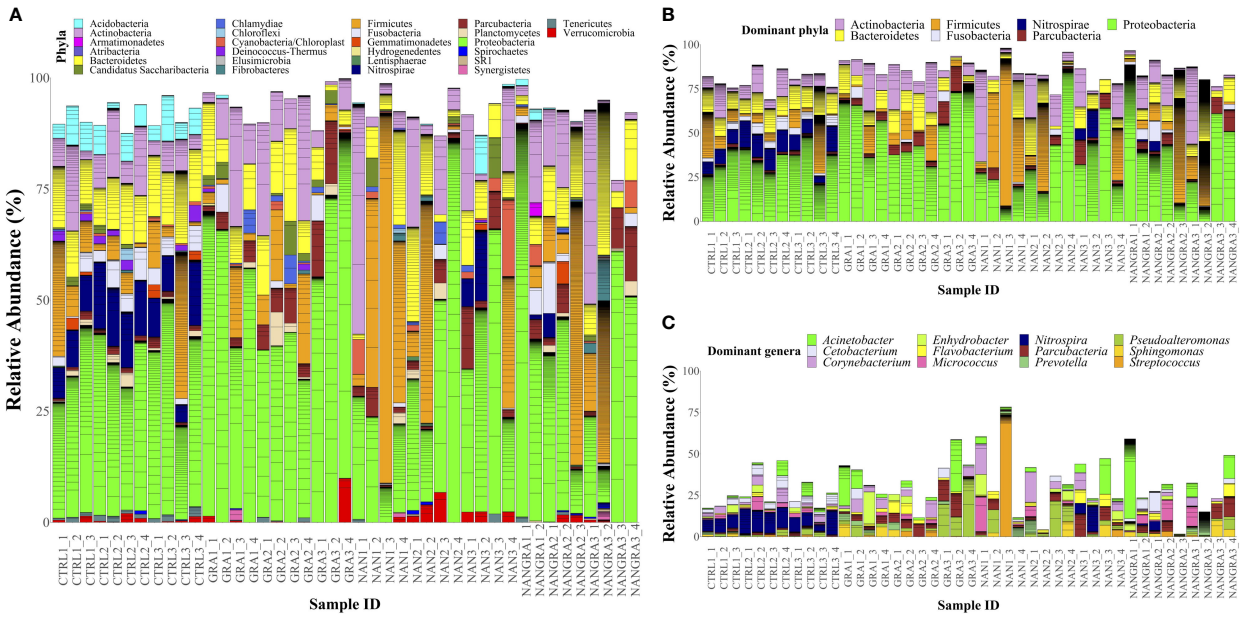

FIGURE 2
Relative abundance of the bacterial phyla **(A)**, dominant phyla **(B)** and dominant genera **(C)** in the posterior intestine of European seabass fed the experimental diets. Sample ID: CTRL, control group; GRA, *Gracilaria gracilis* group; NAN, *Nannochloropsis oceanica* group; NANGRA, *G gracilis* and *N. oceanica* blend group. Color codes: Actinobacteria – pink, Bacteroidetes – yellow, Firmicutes – orange, Fusobacteria – lavender, Nitrospirae – dark blue, Parcubacteria – dark red, Proteobacteria – green. Unclassified bacteria are not shown in the figures.

TABLE 1 Average relative abundance (%) of the dominant bacterial phyla and genera in the posterior intestine of European seabass fed the experimental diets.

	CTRL	GRA	NAN	NANGRA
Phyla				
Actinobacteria	8.34 ± 5.81	11.83 ± 10.11	12.65 ± 15.32	11.16 ± 14.19
Bacteroidetes	12.06 ± 2.25	9.61 ± 5.77	8.79 ± 7.09	11.46 ± 9.63
Firmicutes	8.99 ± 10.66	6.30 ± 7.39	20.98 ± 27.31	14.66 ± 20.10
Fusobacteria	3.84 ± 2.14	1.31 ± 2.41	0.48 ± 0.95	2.75 ± 3.86
Nitrospirae	10.21 ± 3.56	0.002 ± 0.01	1.85 ± 4.79	0.71 ± 1.79
Parcubacteria	1.27 ± 0.60	5.55 ± 4.43	3.79 ± 4.09	4.00 ± 4.45
Proteobacteria	35.25 ± 7.93	53.29 ± 16.57	34.93 ± 22.14	40.45 ± 25.35
Genera				
Acinetobacter	2.27 ± 3.23	7.02 ± 10.92	3.49 ± 5.89	8.23 ± 15.71
Cetobacterium	3.76 ± 2.13	1.31 ± 2.41	0.42 ± 0.97	2.70 ± 3.90
Corynebacterium	2.88 ± 2.79	3.64 ± 3.66	4.95 ± 7.02	1.34 ± 1.75
Enhydrobacter	1.21 ± 1.78	2.64 ± 5.73	2.25 ± 3.82	0.86 ± 1.86
Flavobacterium	0.93 ± 0.70	2.19 ± 2.45	2.39 ± 2.82	1.84 ± 2.57
Micrococcus	2.02 ± 2.24	0.72 ± 1.20	2.47 ± 6.20	3.48 ± 5.21
Nitrospira	10.03 ± 3.36	0.002 ± 0.01	1.77 ± 4.52	0.71 ± 1.79
Parcubacteria	1.27 ± 0.60	5.55 ± 4.43	3.79 ± 4.09	4.00 ± 4.45
Prevotella	0.33 ± 0.51	1.40 ± 1.77	1.25 ± 2.65	1.75 ± 4.40
Pseudoalteromonas	1.26 ± 1.49	7.57 ± 11.18	4.73 ± 6.70	1.63 ± 2.39
Sphingomonas	0.29 ± 0.69	1.60 ± 2.86	1.56 ± 3.18	2.09 ± 3.25
Streptococcus	0.22 ± 0.39	0.75 ± 1.41	6.54 ± 21.20	0.48 ± 1.08

Values presented as mean ± SD.

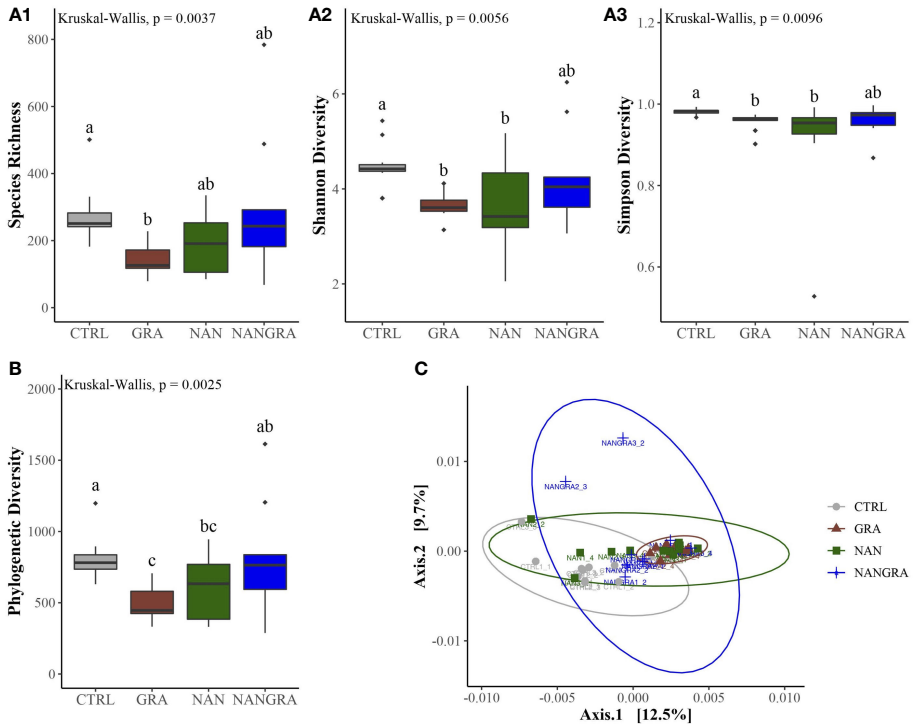

FIGURE 3
Diversity of the bacterial communities of the posterior intestine of European seabass fed the experimental diets. **(A)** Alpha diversity: species richness - A1, Shannon diversity - A2, Simpson diversity - A3; **(B)** Faith's phylogenetic diversity; **(C)** Beta diversity visualized by a principial coordinate analysis (PCoA) plot, by employing weighted UniFrac distances. Different letters denote statistically significant differences ($P < 0.05$) between the dietary treatments (CTRL, control group; GRA, *Gracilaria gracilis* group; NAN, *Nannochloropsis oceanica* group; NANGRA, *G gracilis* and *N. oceanica* blend group).

the community structure. The principal coordinates analysis (PCoA) plot indicated that the first two components explained 12.5 and 9.7% of the variance within the dataset. Results from the PERMANOVA test revealed that the bacterial communities of fish fed the GRA diet were significantly different from those of the CTRL fish ($P = 0.01$) (Figure 3C; F-statistic = 1.932, $R^2 = 0.129$, $P = 0.001$).

Core bacterial taxa of the intestinal microbiota of European seabass

For the core microbial analysis, a prevalence and detection thresholds of 90 and 0.2%, respectively, were concomitantly applied. Results indicated that the core microbial taxa of the posterior intestine of European seabass were composed of three ASVs belonging to genera *Flavobacterium*, *Parcubacteria* and *Lactobacillus* (Figure 4A and Supplementary Figure 2A). When a less restrictive prevalence threshold was applied (i.e., 80%), 7 ASVs belonging to the genera *Flavobacterium* (2), *Parcubacteria* (1), *Lactobacillus* (2), *Cetobacterium* (1) and *Propionibacterium* (1) could be identified as belonging to the common core (Figure 4B and Supplementary Figure 2B).

Modulation of the bacterial communities by the algae diets

The inclusion of macro- and microalgae, included either singly or in combination, in diets for European seabass led to significant alterations on the abundance of several groups of intestinal bacteria compared to the CTRL-fed fish, as evaluated by DESeq2 (Figure 5). The results indicate that the GRA diet, compared to CTRL, led to an increase (10 to 30-fold changes) in the abundance of *Acinetobacter*, *Corynebacterium*, *Ilumatobacter*, *Kordia*, *Methylobacterium*, *Polaribacter*, *Pseudomonas* and *Sulfitobacter*; and a decrease (10 to 30-fold changes) in the abundance of *Bacillus*, *Exiguobacterium*, *Gp4*, *Gp6*, *Nitrospira*, *Opitutus*, *Rhizobium*, *Roseateles* and *Vampirovibrio* (Figure 5A). Concerning the NAN diet, there was an increase in abundance of ASVs of *Acinetobacter*, *Bacillus*, *Kordia*, *Pseudomonas*, *Rothia*, *Streptococcus* and *Thermicanus* when compared to CTRL (20-fold change); while the abundance of

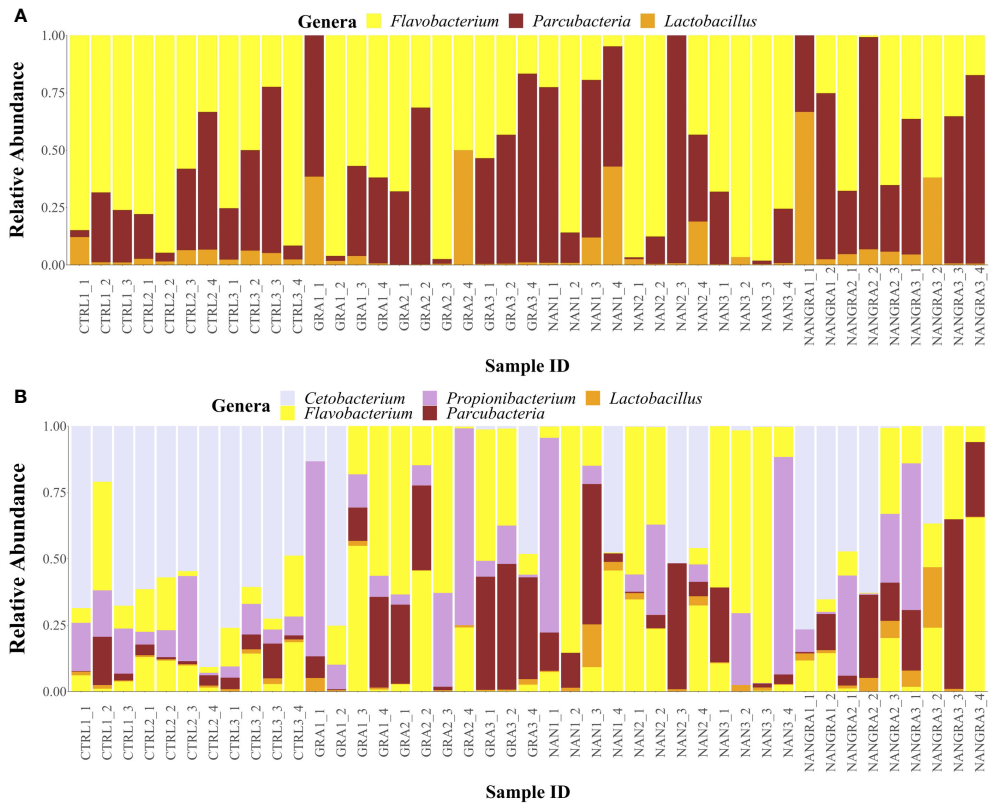

FIGURE 4
Relative abundance of the core bacterial taxa in the posterior intestine of European seabass fed the experimental diets. **(A)** Prevalence threshold of 90%; **(B)** prevalence threshold of 80%. Sample ID: CTRL, control group; GRA, *Gracilaria gracilis* group; NAN, *Nannochloropsis oceanica* group; NANGRA, *G gracilis* and *N. oceanica* blend group.

some members of the *Acinetobacter*, *Clostridium sensu strictu*, *Exiguobacterium*, *Guardnerella*, *Muricauda*, *Ruminobacter*, *Vampirovibrio* and *Vibrio* was decreased, and the fold changes ranged between -20 and -30 (Figure 5B). The inclusion of the blend (NANGRA diet) led to a proliferation (15 to 25-fold changes) of *Acinetobacter*, *Kordia*, *Massilia*, *Parcubacteria* and *Pseudomonas*, and a decreased representation of ASVs of *Clostridium sensu strictu*, *Guardnerella*, *Muricauda*, *Nitrospira*, *Opitutus*, *Pseudomonas* and *Tepidimonas* (-15 to -25-fold changes) (Figure 5C).

Phylogenetic analysis of the ASVs identified as core and those differently modulated by the algae diets

To understand the phylogenetic relationship of certain bacteria which are known to be beneficial and/or pathogenic we first extracted the sequences of the ASVs and performed a blast in NCBI. Thereafter, a phylogenetic tree was constructed to illustrate the genetic relationship, which indirectly indicates functional diversity if there are traits that are retained through evolution.

The phylogenetic tree that includes the ASVs identified as part of the core microbiome in the current study is presented in Figure 6A. The ASVs identified as *Lactobacillus* (ASVs 1679 and 1313) were found to be related to strains of *Lactobacillus delbrueckii* subsp. *lactis*, *Lactobacillus acidophilus*, *Lactobacillus helveticus* and *Lactiplantibacillus plantarum*. The ASV10 *Cetobacterium* clustered with several strains of *Cetobacterium somerae*, while the ASV16 *Propionibacterium* clustered with strains of *Cutibacterium acnes*. The ASVs 11 and 22, identified as *Flavobacterium*, are likely related to a strain of *Flavobacterium succinicans*, whereas the ASV19 *Parcubacteria* clustered with the uncultured candidate division OD1 bacterium.

The phylogenetic relationships of another set of ASVs – i.e., those that were significantly modulated by the dietary treatments – with the highly similar sequences from NCBI are presented in Figure 6B. The *Acinetobacter* ASVs 94 and 288 clustered with strains of *Acinetobacter schindleri*; ASV24 appeared in a cluster with strains of *Acinetobacter baumannii*; and ASV43 clustered with strains of *Acinetobacter pittii*. The ASVs identified as *Pseudomonas* clustered together with species of this genus; *Pseudomonas alcaligenes* (ASV35), *Pseudomonas*

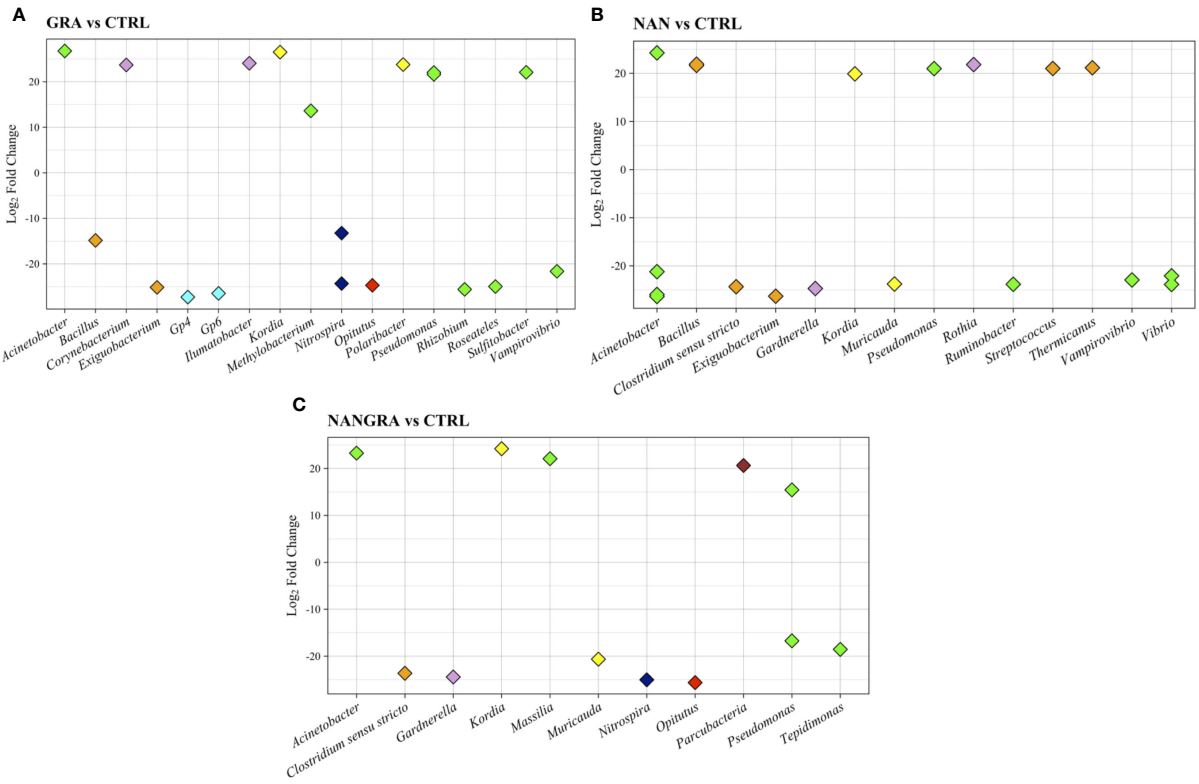

FIGURE 5
Differently abundant genera in the study groups. **(A)** GRA group vs CTRL group. **(B)** NAN group vs CTRL group. **(C)** NANGRA group vs CTRL group. Color codes: green – Proteobacteria (*Acinetobacter*, *Methylobacterium*, *Pseudomonas*, *Rhizobium*, *Roseateles*, *Sulfitobacter*, *Vampirovibrio*, *Ruminobacter*, *Vibrio*, *Massilia* and *Tepidimonas*); orange – Firmicutes (*Bacillus*, *Exiguobacterium*, *Clostridium sensu stricto*); pink – Actinobacteria (*Corynebacterium*, *Ilumatobacter*, *Gardnerella* and *Rothia*); light blue – Acidobacteria (*Gp4* and *Gp6*); yellow – Bacteroidetes (*Kordia* and *Muricauda*); dark blue – Nitrospirae (*Nitrospira*); light red – Verrucomicrobia (*Opitutus*); dark red – *Parcubacteria*.

alcaliphila (ASV37), *Pseudomonas brassicae*, *Pseudomonas putida*, *Pseudomonas japonica* and *Pseudomonas qingdaonensis* (ASV182). *Vibrio* ASV338 clustered with a strain of *Vibrio fortis*; *Ruminobacter* (ASV102) appeared in a cluster with uncultured rumen bacteria; and *Rhizobium* (ASV265) clustered with a strain of *Rhizobium straminoryzae*. *Rothia* (ASV178) is likely related to the strain of *Rothia dentocariosa*; *Streptococcus* (ASV803) clustered with a strain of *Streptococcus parasanguinis*; and the *Bacillus* ASVs clustered with strains of *Geobacillus thermoleovorans* and *Caldibacillus thermoamylovorans* (ASV232) and a strain of *Caldibacillus hisashii* (ASV71). ASV34, annotated as *Kordia*, was phylogenetically close to strains of *Kordia periserrulae* and *Kordia algicida*. *Corynebacterium* (ASV220), *Clostridium sensu stricto* (ASV565), *Exiguobacterium* (ASV240), *Opitutus* (ASV176), *Polaribacter* (ASV113), and *Parcubacteria* (ASV198) clustered with strains of bacteria belonging to the same genera.

Discussion

The gut microbiota of farmed fish has recently become one of the most studied topics in aquaculture research due to the recognized importance of the intestinal bacterial communities to fish health and physiology (Egerton et al., 2018). Although nutrients are known to play a crucial role in modulating the intestine microbiota and algae have been extensively studied in recent years as novel feed ingredients and additives for farmed fish (Hua et al., 2019), the knowledge about the impact of macro- and microalgae on the composition of the intestinal bacteria of fish is still limited (Cerezuela et al., 2012; Rico et al., 2016; Jorge et al., 2019; Keating et al., 2021; Sagaram et al., 2021; Cerezo et al., 2022). In the present study we evaluated the ability of the macroalga *G. gracilis* and the microalga *N. oceanica*, and a mix of these two algae, to modulate the gut bacterial profile of European seabass, a widely farmed fish species in Europe.

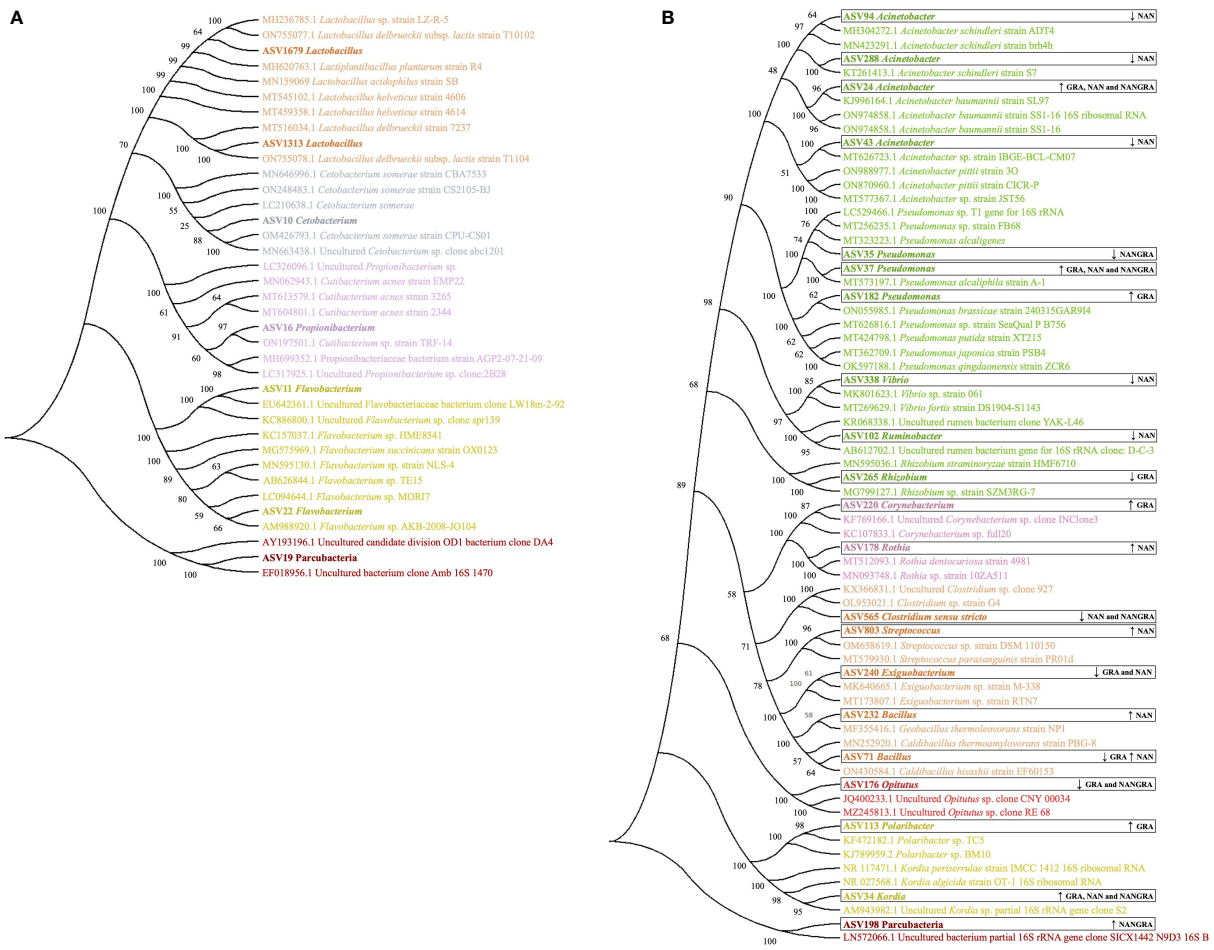

FIGURE 6
Phylogenetic trees generated for the ASVs of interest identified in the study. **(A)** ASVs identified as part of the core microbiome. **(B)** ASVs identified as differently abundant in the study groups. ↑ – increase in abundance; ↓ – decrease in abundance; GRA, *Gracilaria gracilis* group; NAN, *Nannochloropsis oceanica* group; NANGRA, *G gracilis* and *N. oceanica* blend group.

Gut bacterial community composition and diversity in European seabass is affected by the algae diets

The dominant bacterial phyla found in the posterior intestine of European seabass were Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Fusobacteria, Nitrospirae and Parcubacteria. Our findings are in line with previous studies on this fish species that have also reported the dominance of Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Fusobacteria in the gut (Pérez-Pascual et al., 2020; Serra et al., 2021; Liu et al., 2022). The phylum Nitrospirae has a relatively high representation in our samples from the CTRL group (10.21%), and although it has been previously reported as part of the intestinal microbiota of farmed fish, it was not found among the most abundant phyla in other fish microbiome studies (Li et al., 2019; Serra et al., 2021). As sulfate reducing bacteria, Nitrospirae are usually associated with the water and biofilters of RAS (Schmidt et al., 2016; Minich et al., 2020; Fossmark et al., 2021). Therefore, the presence in the intestine of cultured fish is most likely the result of a transfer event, as also reported by Minich et al. (2020) in Atlantic salmon reared in RAS systems. We did not observe a clear dominance of certain genera across all experimental samples, but *Acinetobacter, Nitrospira*, *Pseudoalteromonas* and *Streptococcus* were among the most abundant genera. The genera *Acinetobacter* and *Streptococcus* spp. have strains with probiotic effects (Swain et al., 2009; Bunnoy et al., 2019), but some species are opportunistic bacterial pathogens that cause diseases in immunocompromised fish or hosts that encounter environmental stressors (Dawood, 2020). On the other hand, *Pseudoalteromonas* species can provide protection against harmful bacteria present in the gut microbiota, namely *Vibrio* (Richards et al., 2017; Rimoldi et al., 2020). The relatively high abundance of both potentially pathogenic and beneficial bacteria found in the fish intestine denotes the importance of the commensal microbiota in

the maintenance of host health, through a tight regulation of competing microorganisms.

The bacterial alpha diversity analysis revealed that the 8% inclusion of *G. gracilis* or *N. oceanica* reduced the species richness (significant only for *G. gracilis*), evenness of the microbial populations and the effective number of dominant species, compared to the CTRL group. Similarly, Faith's phylogenetic diversity, that is a measure of biodiversity based on phylogeny, was also significantly lower in single algae groups. The gut microbial community of European seabass that consumed the seaweed supplemented diet (GRA) also differed significantly compared to the CTRL-fed fish. Although a decreased bacterial diversity may not always imply an unstable community, a reduction in the microbial diversity and changes in the bacterial profiles in the intestine have been associated with unhealthy fish (Li et al., 2016). Interestingly, inclusion of the algae blend eliminated these negative effects on the intestinal bacterial diversity, most probably due to the lower inclusion (4%) of each alga. In a complementary study (Batista et al., 2020), we evaluated the effects of *G. gracilis* and *N. oceanica* on growth performance, nutrient digestibility and intestinal histomorphology; a lower protein digestibility was observed for fish fed the macroalga supplemented diet, but without differences in terms of growth. The fish on this diet had the lowest number of intestinal neutral goblet cells associated with the digestive and absorptive processes. Similar to the ability bestowed by the microalga in the *G. gracilis-N. oceanica* blend to regain the diversity and structure of the intestinal microbiota, our previous study also indicated the positive association of the microalga with the digestion process (Batista et al., 2020). Together, these results may indicate that inclusion of different micro- and macroalgae in a blend is likely a good strategy to lessen the negative impacts of added-value natural compounds from macroalgae species.

Core microbiome of European seabass

The core microbiota (autochthonous microbiome) is composed of resident microorganisms that colonize the intestinal mucosa (Egerton et al., 2018). In the present study, three and five genera were identified as part of the core microbiota at 90 and 80% prevalence thresholds, respectively. The core bacteria were *Flavobacterium*, *Lactobacillus*, *Parcubacteria*, *Cetobacterium* and *Propionibacterium*. Although part of normal fish microbiota, the genus *Flavobacterium* is well recognized for their opportunistic nature in fish (Derome et al., 2016). Some *Flavobacterium* spp. are responsible for systemic infections that are difficult to control and prevent, and hence, are associated with devastating economic losses that affect the worldwide fish production (Loch and Faisal, 2015). In the present study, the *Flavobacterium* ASVs identified as part of the core microbiome showed a high similarity with a potentially pathogenic bacteria, *F. succinicans*, the likely agent of bacterial gill disease in rainbow trout cultivated in RAS (Good et al., 2015). Interestingly, the genus *Lactobacillus*, belonging to the lactic acid bacteria group, was also identified as part of the core microbiota of European seabass in our study. *Lactobacillus*, well recognized as part of the beneficial gut bacteria, provides protective effects against bacterial infections (He et al., 2017; Dawood, 2020). The core ASVs belonging to *Lactobacillus* clustered with *L. delbrueckii* subsp. *lactis*, *L. acidophilus*, *L. helveticus*, and *Lactiplantibacillus plantarum*, that were reported to have *in vitro* and *in vivo* probiotic activity (Rurangwa et al., 2009; Hosseini et al., 2016; Ahire et al., 2019; Iorizzo et al., 2022).

ASVs assigned to *Parcubacteria* were detected among both core microbiome and the top ASVs in our samples (between 1.3 and 5.6% average relative abundance). Genes encoding for amylases and capacities for degrading cellulose and mannose were associated with *Parcubacteria* isolated from sporadic permafrost zone of subarctic Quebec, and this bacteria has the capacity to produce acetate as the major end product of its metabolism (Vigneron et al., 2019). Nonetheless, the role of *Parcubacteria* as part as the gut microbiota of European seabass remains unclear and further studies should delve into the functionality of this bacteria.

As part of the commensal microbiota, *Cetobacterium* and *Propionibacterium* are also often described as beneficial bacteria that are commonly found in healthy fish. Here, the ASVs identified as core and belonging to *Cetobacterium* and *Propionibacterium* were closely related to the strains of *C. somerae* and *Cutibacterium acnes*, respectively. *Cetobacterium* is reported as protease and vitamin B_{12} producing bacteria (Legrand et al., 2019), and previous studies have demonstrated the ability of the fermentation products of *C. somerae* to improve gut health of different farmed fishes (Xie et al., 2021; Xie et al., 2022; Zhou et al., 2022). The genus *Propionibacterium* - producer of propionate, linolenic acid, vitamins, and antimicrobials - is known to enhance host robustness by boosting the immune response and promoting the growth of other probiotic bacteria (Zárate, 2012; Boutin et al., 2013). In gilthead seabream *Propionibacterium* has also been reported as an important genus of the core intestinal microbiota, producing metabolites with an important role in gut health (Piazzon et al., 2017; Piazzon et al., 2019).

The presence of taxa, namely *Lactobacillus* and *Cetobacterium*, with probiotic potential among the highly prevalent taxa in majority of the studied population indicate that these bacteria play a key role in the maintenance of biological functions, health, and disease resistance in European seabass.

Algae, singly or in combination, modulate both beneficial and potentially pathogenic groups of bacteria

The intestinal microbiota of farmed fish is a complex assemblage of microorganisms that interact and compete for

nutrient acquisition and space. Both beneficial and potentially pathogenic groups of bacteria coexist in the gut, with diet playing a leading role in modulating these bacterial communities (Tarnecki et al., 2017; Dawood, 2020). In the present study, macro- and microalgae feeds affected the abundance of several groups of bacteria present in the posterior intestine of seabass when compared to fish fed the CTRL diet.

The inclusion of *G. gracilis* and *N. oceanica*, fed both singly and blended, led to a significant increase in the representation of ASVs belonging to potentially pathogenic bacteria. Notably there was an increased representation of an ASV that clustered with strains of *Acinetobacter baumannii*; a strain of this bacterium was previously recovered from diseased channel catfish (*Ictalurus punctatus*) (Xia et al., 2008). The GRA diet led to an increase in a *Pseudomonas* ASV that clustered together with *P. putida*, a bacteria known to cause disease in both humans and fish (Mao et al., 2013). *Rothia*, that proliferated in the NAN group, has only been reported as an opportunistic pathogen in humans (Fatahi-Bafghi, 2021). As fish may act as a vehicle for the transmission of drug-resistant human pathogens, it is of crucial importance to understand the impact of novel formulations on the fish microbial communities. Other groups of potentially pathogenic bacteria that were significantly increased only in the gut of GRA-fed fish included *Corynebacterium* (Tarnecki et al., 2018) and *Polaribacter*, a potential RAS-associated pathogen (Rud et al., 2017). It is noteworthy that some *Polaribacter* species are associated with important antioxidant functions (Sehnal et al., 2021). A pro-oxidative or bacterial challenge could give further insights into the impact of these potentially pathogenic genera on fish health.

The *Gracilaria* diets (GRA and NANGRA) were associated with a significant decrease of the genus *Nitrospira*. During the 15-weeks feeding trial, all abiotic conditions, including the nitrogenous compounds in the RAS system, were carefully monitored, and optimum water quality parameters were maintained in the rearing system of the fish. Therefore, the lower proportion of the genus *Nitrospira* (the phylum Nitrospirae) found in macroalgae groups is most likely a response to the dietary treatment. Suo et al. (2017) studied the impact of sulfide exposure on gut microbiota of Pacific white shrimp (*Litopenaeus vannamei*) and reported that although present in the intestine of non-exposed shrimp, Nitrospirae could not be found in sulfide-exposed groups. The authors argued that the disappearance of this bacterial phylum upon sulfide exposure could make shrimp more susceptible to nitrite toxicity. *Nitrospira* spp. are nitrite-oxidizing bacteria that convert nitrite into the less toxic nitrate (Philips et al., 2002). Therefore, the reduction of *Nitrospira* in intestine of European seabass fed *G. gracilis* diets might negatively impact the ability of the fish to cope with a nitrite exposure situation. Nitrite uptake in marine fish is thought to occur through the gills and intestine, and although it is less toxic for marine fish, previous studies have found negative impacts of elevated nitrite levels on growth (Ciji and Akhtar, 2019).

The algae-rich diets modulated certain groups of bacteria involved in nutrient degradation and utilization. The genus *Opitutus*, that are cellulose degraders commonly found on surfaces of green periphytic algae (Knack et al., 2015), was less abundant in seaweed-rich diets. Likewise, a decreased abundance of bacteria belonging to the genus *Rhizobium* that are known to have cellulolytic and pectolytic activity (Xia et al., 2018), was observed in the GRA group. Macroalgae, much like plants, have cellulose-based walls (Kumar et al., 2013). Therefore, these bacterial groups may aid in the degradation of the algae and plant products present in the experimental diets, and a decrease in its abundance may partially explain the lower nutrient digestibility associated with the *Gracilaria* diets, as reported by Batista et al. (2020). Indeed, the authors attributed the reduced protein and energy digestibility in fish fed the GRA and NANGRA diets to the presence of indigestible fibers in the macroalga cell wall that compromised the action of the digestive enzymes. The involvement of the gut microbiota in the digestive/absorptive processes in fish has been previously recognized (Butt and Volkoff, 2019), and the above-mentioned results further support these interactions. Although the genera *Ruminobacter*, described as starch utilizer (Darabighane et al., 2021), and *Clostridium sensu stricto*, previously characterized as a carbohydrate degrader (Abdelhamed et al., 2019), had a lower abundance in the intestine of fish fed the *Nannochloropsis* diet, in our associated study (Batista et al., 2020) we did not find a difference in nutrient or energy digestibility in the NAN group compared to the CTRL group. Moreover, fish fed the algae supplemented diets had an increased representation of an ASV that was annotated as *Kordia*, that is likely closely related to *K. periserrulae* and *K. algicida*. Members of *K. algicida* can exhibit algicidal activity and produce extracellular proteases responsible for the cell lysis of diatoms in a species-specific way (Demuez et al., 2015). Therefore, a higher abundance of *Kordia* in the intestine of European seabass may aid in the digestion of microalgae enriched diets. On the other hand, the genus *Exiguobacterium* was found to be less abundant in not only the NAN-fed fish but also the GRA-fed fish. A previous study on zebrafish has found that *Exiguobacterium* sp. play a role in lipid droplet formation in enterocytes to positively impact the fatty acid absorption (Semova et al., 2012). Further studies on the function of this bacterial genus on fish gut microbiota are required, but the reduced abundance of *Exiguobacterium* in single alga diets may indicate an impact of these algae on the metabolism of dietary fat. Nonetheless, there were no differences between the lipid digestibility and whole-body and muscle fat contents of fish that consumed algae-rich diets and those fed the CTRL diet (Batista et al., 2020).

A modulation of both beneficial (i.e., increase) and potentially pathogenic (i.e., decrease) bacteria was observed in

the intestine of European seabass fed the macro- or microalgae diets. Inclusion of seaweed *G. gracilis* in feeds led to a higher abundance of *Sulfitobacter* and *Methylobacterium* (producer of poly-β-hydroxybutyrate that degrades short-chain fatty acids), two genera that comprise bacteria capable of inhibiting the growth of fish pathogens (Boutin et al., 2013; Wilczynski et al., 2022). Similarly, the *N. oceanica* feeds led to an increase in beneficial bacteria such as *Bacillus* in fish intestine. Spore-forming species such as *Bacillus* spp. are probiotics with wide application in the aquaculture industry. The *Bacillus* ASVs that were enriched in the gut of NAN-fed fish clustered together in the phylogenetic tree with bacterial strains such as *Geobacillus thermoleovorans*, *Caldibacillus thermoamylovorans* and *C. hisashii* that exhibit antimicrobial and probiotic activities (Inabu et al., 2022; Zebrowska et al., 2022). Spore-forming bacterial species belonging to the genus *Bacillus* have been previously isolated from the intestine of European seabass, with some isolates presenting carbohydrase activity and probiotic potential (Serra et al., 2019). From an industrial perspective, it would be interesting to further explore the ability of the microalga *N. oceanica* to enrich the gut of European seabass with *Bacillus* spp. with promising probiotic activity. Although limited information on the topic is available, some studies have reported that species belonging to the genus *Acinetobacter*, namely *Acinetobacter lwoffii*, *Acinetobacter junii* and *A. pittii* are emerging fish pathogens and their virulence against fish of the genus *Schizothorax* was also revealed previously (Cao et al., 2018; Malick et al., 2020). Interestingly, the NAN diet led to a decrease in several *Acinetobacter* ASVs, including an ASV that likely has a close phylogenetic relationship with the pathogen *A. pittii*. Although the genus *Pseudomonas* is often reported as part of the normal microbiota and certain bacteria belonging to this taxa are probiotic strains (Qi et al., 2020), there are also opportunistic fish pathogens among them that are responsible for disease outbreaks and high mortality in farms (Oh et al., 2019). NANGRA-fed fish presented a reduced abundance of an ASV belonging to *Pseudomonas* that is probably related to *P. alcaligenes*, a rare but potentially opportunistic fish pathogen (Bai et al., 2021). This microalga also decreased the abundance of the genus *Vibrio*, one of the most important fish pathogens responsible for devastating economic losses in fish farms. In the present study, the ASV of the genus *Vibrio* clustered with a strain of *Vibrio fortis*, a bacterium with reported pathogenicity against rainbow trout (Austin et al., 2005), and that has been found in association with enteritis in cultured seahorses (*Hippocampus erectus*) (Wang et al., 2016). *In vitro* studies have recently demonstrated the antimicrobial activity of *G. gracilis* and *N. oceanica* against some *Vibrio* species, namely *V. harveyi* and *V. parahaemolyticus* (Ferreira et al., 2021). Disease outbreaks caused by *Vibrio* are bottlenecks for the long-term sustainability of the aquaculture sector. In this context, microbiota modulation using added-value products such as those employed in this study may be a promising strategy to improve disease resistance in fishes.

Conclusion

In the present study it was demonstrated for the first time the potential of the seaweed *G. gracilis* and the microalga *N. oceanica*, incorporated singly or blended in European seabass diets, to modulate the intestinal microbiota of the fish. It was observed that 8% inclusion of the two algae (singly) led to a reduction of the gut microbial diversity, which is often associated with a negative impact. Nonetheless, inclusion of a lower percentage of each alga in a blend (4% each) was able to mitigate these plausible effects on the intestinal bacterial communities. The core microbiome of European seabass was composed of both beneficial (*Lactobacillus* and *Cetobacterium*) and potentially pathogenic (*Flavobacterium*) bacteria, which might suggest that the host gut homeostasis and disease resistance is dependent on a tight interaction between competing microorganisms. The algae-rich feeds modulated some groups of bacteria that are known carbohydrate degraders, with the *Gracilaria*-fed fish showing a decreased abundance of the genera *Opitutus* and *Rhizobium*, which may partially explain the lower nutrient digestibility observed in fish that consumed the macroalgae diet. *Gracilaria* promoted the growth of bacteria capable of outcompeting fish pathogens (*Sulfitobacter* and *Methylobacterium*). *Nannochloropsis*, on the other hand, led to a higher representation of *Bacillus* bacteria, widely recognized for their probiotic potential, and a decreased abundance of the potentially pathogenic bacteria belonging to the genus *Acinetobacter*. *Nannochloropsis*-fed fish also presented a lower abundance of ASVs identified as *Vibrio*, a highly pathogenic bacteria that affect the culture of several farmed aquatic animals of high economic value. Such results are indicative of the potential of algae to modulate the bacterial communities present in the intestine of European seabass, with possible implications to host health, nutrition, and disease resistance.

Data availability statement

The datasets presented in this study are deposited in the NCBI repository (https://www.ncbi.nlm.nih.gov), accession number PRJNA867546.

Ethics statement

This study was reviewed and approved by Review of the ethical process concerning all animal handling and sampling procedures performed by CIIMAR (Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal) animal welfare body (ORBEA-CIIMAR) and approved by national competent authorities.

Author contributions

LV and VK were responsible for funding acquisition and the conceptualization of the study. MF and YA performed the formal analysis and investigation. HA and JS provided the resources for the study. MF wrote the original draft under the supervision of LV and VK, who reviewed and edited it. All authors contributed to the article and approved the submitted version.

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Conflict of interest

HA and JS were employed by the companies ALGAplus and Allmicroalgae, respectively.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1001942/full#supplementary-material

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