Biostimulation of *in situ* microbial degradation processes in organically-enriched sediments mitigates the impact of aquaculture

Francesca Ape [a], Elena Manini [b], Grazia Marina Quero [c], Gian Marco Luna [b], Gianluca Sarà [d], Paolo Vecchio [e], Pierlorenzo Brignoli [e], Sante Ansferri [e], Simone Mirto [a, *]

[a] Istituto per lo studio degli impatti Antropici e Sostenibilità in ambiente marino (IAS-CNR), Via G. da Verrazzano, 17, 91014, Castellammare del Golfo, TP, Italy
[b] Istituto per le Risorse Biologiche e le Biotecnologie Marine (IRBIM-CNR), Via Largo Fiera della Pesca, 1 – 60122 Ancona, Italy
[c] Stazione Zoologica Anton Dohrn, Integrative Marine Ecology Department, 80121, Napoli, Italy
[d] Dipartimento di Scienze della Terra e del Mare, University of Palermo, Viale delle Scienze Ed. 16, 90128, Palermo, Italy
[e] Eurovix S.p.A. - V.le E. Mattei 17, 24060, Entratico (Bergamo), Italy

HIGHLIGHTS

- Bioremediation reduces the accumulation of organic matter in fish farm sediments.
- The bioactivator stimulates *in situ* microbial degradation processes.
- Bioremediation induces shifts in prokaryotic community composition.
- A shift from anaerobic to aerobic prokaryotic metabolism is promoted.
- The treatment is ineffective on the fecal bacteria from farmed fishes.

GRAPHICAL ABSTRACT
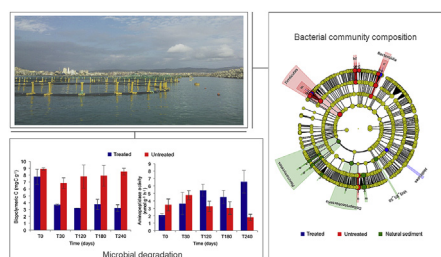
ABSTRACT

Fish farm deposition, resulting in organic matter accumulation on bottom sediments, has been identified as among the main phenomena causing negative environmental impacts in aquaculture. An in *situ* bioremediation treatment was carried out in order to reduce the organic matter accumulation in the fish farm sediments by promoting the natural microbial biodegradation processes. To assess the effect of the treatment, the concentration of organic matter in the sediment and its microbial degradation, as well as the response of the benthic prokaryotic community, were investigated. The results showed a significant effect of the treatment in stimulating microbial degradation rates, and the consequent decrease in the concentration of biochemical components beneath the cages during the treatment. During the bioremediation process, the prokaryotic community in the fish farm sediment responded to the overall improvement of the sediment conditions by showing the decrease of certain anaerobic taxa (e.g. Clostridiales, Acidaminobacteraceae and Caldilineaceae). This suggested that the bioactivator was effective in promoting a shift from an anaerobic to an aerobic metabolism in the prokaryotic community. However, the larger importance of Lachnospiraceae (members of the gut and faecal microbiota of the farmed fishes) in treated compared to non-treated sediments suggested that the bioactivator was not efficient in reducing the accumulation of faecal bacteria from the farmed fishes. Our results indicate that bioremediation is a promising tool to mitigate the aquaculture impact in fish farm sediments, and that further

---

* Corresponding author.
E-mail address: simone.mirto@cnr.it (S. Mirto).

1. Introduction

Since 2014, aquaculture has provided more fish for human consumption than capture fisheries, and it is expected to provide up to 60% of the fish available for human consumption by 2030 (FAO, 2018). Fish farming production in the Mediterranean sea, particularly that regarding sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*), has increased by 77% over the last decade, especially in coastal systems (Neofitou et al., 2010; Rosa et al., 2012). The rapid expansion of Mediterranean aquaculture activities in coastal areas, facilitated by the environmental characteristics of the zone (FAO, 2008), has drawn an increasing concern on their environmental impact (Bouwman et al., 2013), since a rapid dispersion of farming-related wastes into the surrounding waters, mainly represented by organic matter (i.e. uneaten food and faeces), nutrients and pharmaceutical products (i.e. antibiotics) occurs (Bouwman et al., 2013).

Increased organic inputs negatively affects marine sediments, producing changes in the structure of the benthic communities and oxygen depletion, and eventually leading to anoxic sediments as the excess of organic material decomposes on the sea floor (Klaoudatos et al., 2006; Neofitou et al., 2010; Mirto et al., 2012). Benthic assemblages in proximity of aquaculture farms exhibit signs of disturbance, potentially affecting the whole food web and leading to an overall decrease in biodiversity (Karakassis et al., 2000). In light of this, the attention on the use of bioremediation strategies in fish farm impacted areas is increasing (Yu et al., 2014; Robinson et al., 2016; Casado-Coy et al., 2017). Typically, most of the environmental bioremediation interventions have been aimed to the recovery of terrestrial and marine areas impacted by chemical pollution due to accidental spills (mostly by hydrocarbons) or to urban and industrial discharges (Suzuki et al., 2002; Genovese et al., 2014). Nevertheless, the recovery of organically enriched sediments (such as those in proximity of aquaculture farms) by means of bioremediation has received less attention. In particular, the potential bioremediation actions by means of the metabolic activity of marine prokaryotes have been, so far, poorly explored (Vezzulli et al., 2004; Wang et al., 2007).

To date, the most used strategies to reduce the excess in nutrients released from aquaculture activities in coastal ecosystems have focused on the contextual farming of bivalves and seaweed (Huo et al., 2012; Wu et al., 2015), due to capacity of marine algae to bioaccumulate the nutrients produced by fish farms (Sanderson et al., 2012; Yu et al., 2014). New strategies have satisfactorily used the addition of compost to treat organically enriched sediments and to increase the degradation rates of organic matter by *in situ* prokaryotic communities (d'Errico et al., 2013). Other studies suggested the introduction of colonies of polychaetes (*Capitella* sp.) and of the sea cucumber *Holothuria scabra* (Robinson et al., 2016) to accelerate the decomposition of the organic matter in fish farm sediments, also through the stimulation of microbial activities (Wada et al., 2005; Kunihiro et al., 2011).

The exploitation of the natural metabolic potential by benthic microbes is accepted as an environmentally benign and economic measure for decontamination of polluted environments (Wu et al., 2015). In fact, benthic prokaryotes are known to play a crucial role in the degradation processes of sedimentary organic matter and its transfer to higher trophic levels (Manini et al., 2003; Reimers et al., 2013). Several strains of bacteria isolated from the environment have been shown to be capable of removing N, P and sulfur compounds, and to be potentially useful in bioremediation applications (Guo et al., 2013; Zhang et al., 2015; Zhang et al., 2018; 2019). However, practical applications aimed at bioremediating organically enriched sediments are still scarce (Dell'Anno et al., 2009; Zhang and Lo, 2015), prompting the need to undertake studies to test *in situ* the efficacy of these alternative strategies in mitigating the environmental consequences of aquaculture.

In the present study, we investigated the effectiveness and the effect of a bioremediation treatment on the organically enriched sediments in a fish farm located in the Mediterranean Sea, by adding a bioactivator that promotes the natural biodegradation processes of sedimentary organic matter. We monitored (over a 32 weeks period) the effects of the bioactivator on the degradation and fate of the sediment organic matter, and investigated the response of prokaryotic community as total abundance and biomass, community composition and main degradation activities of the organic detritus.

2. Materials and methods

2.1. Study site

The bioremediation experiment was carried out between October 2014 and July 2015, in a marine fish farm located in the harbour of Licata (Southern Sicily, Mediterranean Sea; coordinates 37.087713° N, 13.943773° E. The farm covers a surface of ~8000 m^2 and is composed of 23 floating cages arranged in two rows, containing sea bass (*Dicentrarchus labrax*) and sea bream (*Spaurus aurata*) with a total annual production exceeding 300 tons. The farm, in operation from 1994, is located in a semi-enclosed and sheltered area, characterized by a limited hydrodynamic circulation and a shallow depth (~10 m). Consequently, a large amount of organic matter, in the form of uneaten food and faeces of the reared fish, accumulates on the sea floor under the cages, a phenomenon that seems to cause a progressive transformation of the benthic substrate into a muddy black sediment. During the year, the fish farm area is affected by severe hypoxia phenomena, especially in the period from August to October, when the concentration of oxygen in the water column can often drop to as low as ca. 2 mg l^{-1}. This makes the fish farm an optimal site to investigate the potential effect of bioremediation strategies. During the sampling period, seawater temperature ranged from 15 °C to 24 °C (in March and in July, respectively), while the dissolved oxygen values were constant above 6.1 mg L^{-1}.

2.2. Experimental design and sampling

The *in situ* bioremediation experiment lasted 32 weeks. The treatment consisted in supplying weekly the sediment with a commercially available bioactivator (MICROPAN Aquacombi, a complex mixture of microbes and molecules) inside an area of 800 m^2 below the fish cages (Fig. 1). Sampling was carried out before the treatment (T0) in October and after 30, 120, 180 and 240 days (T30, T120, T180 and T240, respectively) from the beginning of

Fig. 1. Sampling stations inside the harbour of Licata (Southern Sicily). Two COMBI stations below 2 cages were sited inside the treated area (COMBI1 and COMBI2), one aquaculture control station (CTRL1) was below one untreated cage inside the fish farm, and one external control station (CTRL 2) was located far from the farm.

the bioremediation treatment. Sediment samples were collected at 3 stations located at ~10 m below the cages containing sea bass (volume ca. 2000 m^3). Two stations were located inside the treated area (COMBI1 and COMBI2) and one untreated station (CTRL 1) was used as control to investigate the effects of bioremediation on the fish farm sediment (Fig. 1). Additional samples were also collected from an external control station (CTRL 2), located at ~700 m distance from the fish farm, in proximity of the opening of the harbour, and that, as previously assessed, was not influenced by the farm. Sediment samples (0–10 cm) were collected by SCUBA divers. At each station, sediment for the analyses of biochemical components of organic matter (protein, carbohydrate and lipid concentrations) were collected, in triplicate, using corers (diameter 3.6 cm). Sterile corers were used for sediment intended for the analyses of microbial parameters. The samples were immediately transported to the laboratory, where the top 1 cm of each corer was carefully extruded and stored at −20 °C for organic matter, prokaryotic abundance and diversity analyses. For the measurements of the microbial extracellular enzymatic activities, aliquots of freshly-collected sediment, taken from the sediment core (top 1 cm), were used to prepare a sediment slurry using 1:1 dilution (vol/vol) in 0.2 μm prefiltered seawater collected at the water-sediment interface from each station, and further analysed as described below.

2.3. Characteristics of the bioactivator

The commercial product MICROPAN Aquacombi, whose formulation is provided by Eurovix SpA (Brescia, Italy), is a bio-activator consisting in tablets (diameter 8 cm) which contain a complex mixture of vegetable extracts (used as support), a natural enzymatic component, mineral biocatalysts (rich in oligoelements), selected extracts of yeast, selected microorganisms from controlled fermentation, bacterial growing factors, carbohydrates and an active principle from *Fucus laminariae*. A similar bioactivator has been recently proven to be highly effective in removing organic pollutants from a contaminated burned woodland soil (Andreolli et al., 2015). The formula of Aquacombi has been studied to

facilitate the specific application in aquatic systems, and is especially indicated for the treatment of anoxic sediments. Bioremediation was performed in the selected area (800 m^2) located inside the fish farm. The tablets, formulated to dissolve at slow release, were distributed manually every week for a total of 32 weeks (at the rate of 156 g per 100 m^2 of treated areas) in the seawater immediately below the cages. To investigate the improvement of the quality of fish farm sediments, the redox potential discontinuity (RPD) depth was visually estimated as the depth at which sediment colour turns from brown to black.

2.4. Biochemical composition of organic matter

Proteins, carbohydrates and lipids were analysed in triplicate on sediment samples collected from all stations in each sampling period according to Fabiano and Danovaro (1994), and reported also as Biopolymeric Carbon Content (BPC), as sum of lipid, protein and carbohydrate carbon (Pusceddu et al., 2003). We used protein contribution (PRT) to BPC (PRT/BPC%) and the values of the protein to carbohydrate (CHO) ratio (PRT:CHO) as descriptors of the aging and nutritional quality of organic matter in the sediment (Pusceddu et al., 2010).

2.5. Extracellular enzymatic activities

Extracellular enzymatic activities (aminopeptidase [MCA], β-glucosidase [Glu-MUF] and alkaline phosphatase [MUF-P]), in the surface sediment layer (0–1 cm) were determined by the analysis of the cleavage rates of fluorogenic substrates (L-leucine-4-methylcoumarinyl-7-amide, 4-MUF-b-D-glucopyranoside, and 4-MUF-Pphosphate, respectively; SIGMA; Hoppe, 1993), as described in Danovaro et al. (2002). Samples were centrifuged (800×g, 5 min) and the fluorescence of supernatants was measured fluorometrically (at 380 nm excitation, 440 nm emission for MCA and 365 nm excitation, 455 nm emission for Glu-MUF and MUF-P), using a Cary Eclipse spectrofluorometer, immediately after the addition of the substrate and after the incubation (in the dark, at *in situ* temperature for 1 h). Fluorescence was converted into

enzymatic activity by using appropriate standard curves. Enzymatic activities in sediments were normalized to sediment dry weight (60 °C, 24 h) and expressed as nmol of substrate hydrolysed g^{-1} of sediment dry weight h^{-1}.

2.6. Prokaryotic abundance, biomass and diversity

Total prokaryotic abundance in all samples was quantified as described by Manini et al. (2003). Subsamples were 500-folds diluted, filtered onto black Nuclepore 0.2-μm-pore-size filters. and analysed using epifluorescence microscopy (1000 × magnification). For the calculation of the total prokaryotic biomass (TPB), 20 fg of C cell^{-1} were used as the typical conversion factor in marine microbiology studies (Cho and Azam, 1990). Total prokaryotic abundance and biomass were normalized to sediment dry weight after desiccation (24 h at 60 °C).

To analyse diversity, prokaryotic DNA was extracted from 1 g of each sediment sample using the PowerSoil® DNA Isolation Kit (MoBio Laboratories Inc., California), by following the manufacturer's instructions with some slight modifications to increase the DNA yield and quality (Quero et al., 2017). These included two more vortexing steps (following the one that is recommended by the manufacturer) at the maximum speed for 2 min, each one being preceded by an incubation step at 70 °C for 5 min, and with the addition of one more washing step with Solution C5 as an additional removal step for contaminants. The concentration of each DNA extract was determined using a Qubit Fluorometer (Thermo-Fisher), and the DNA was then stored at −80 °C until PCR. Illumina Miseq sequencing analyses were carried out on the hypervariable V3 and V4 regions of the 16S rRNA gene by amplifying using the 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GAC-TACHVGGGTATCTAATCC-3′) universal bacterial primers (Eiler et al., 2012). Paired-end reads were quality checked (with default settings and minimum quality score of 20) and analysed with QIIME v1.8.0 software package (Quantitative Insights Into Microbial Ecology). Reads were clustered into OTUs by using UCLUST v1.2.22 (Edgar, 2010) with a >97% similarity threshold with an open-reference OTU picking strategy and default settings. Chimeras were detected by using USEARCH v6.1 (Edgar, 2010). Chimera checking and taxonomy assignment was performed using Greengenes 13.8 as reference database (DeSantis et al., 2006). Abundances in each sample were normalized to the number of sequences of the sample with the lowest number of reads retained. The sequences are submitted to the SRA – Sequence Read Archive (BioProject PRJNA525837).

2.7. Statistical analysis

Univariate and multivariate distance-based permutational nonparametric analyses of variance (PERMANOVA; Anderson, 2001; McArdle and Anderson, 2001) was performed to compare the concentration of the different biochemical components of organic matter, extracellular enzymatic activities, abundance, biomass and richness of prokaryotes between treated stations (referred to as COMBI), untreated station (CTRL1) in the fish farm and the external control station (CTRL2) (treatment as fixed factor with 3 levels). To determine the differences in the same parameters in each station before (T0) and during 32-week bioremediation (after 30, 120, 180 and 240 days after the start of the treatment), we considered the experimental time as fixed factor with 5 levels. PERMANOVAs were based on Euclidean distance matrices, calculated on normalized data for organic matter variables, and Bray−Curtis similarity matrix for abundance data after log(x+1) transformation, using 9999 random permutations of the

appropriate units (Anderson, 2001). When significant differences were observed, post-hoc pairwise tests were performed to test for the differences between the stations before and during the treatment and in each station among the different experimental times for each investigated parameters.

Linear Discriminant Analysis (LDA) Effect Size (LEfSe) method (Segata et al., 2011) was applied to 16S rRNA biomarkers to identify which of the bacterial taxa significantly explained the differences in community composition between treatments (COMBI, CTRL1 and CTRL2).

3. Results

3.1. Biochemical composition of organic matter

For all the investigated variables, due to the similarity of the results, we displayed the average of the data obtained from the sediments collected from the two treated stations (COMBI1 and COMBI2), referring to them as "COMBI".

The RPD depth in the sediments was higher in the control site (~3 cm) than in the fish farm sediment (COMBI and CTRL1), where we typically found black sediment starting from the first centimetre. Visually, this condition appeared to be permanent during the experiment.

The investigated stations (COMBI, CTRL1 and CTRL2) displayed clear differences in terms of quantity of the biochemical components of organic matter (PERMANOVA results in Supplementary material; Table S1). The concentration of proteins, lipids, carbohydrates and biopolymeric organic carbon before the treatment (T0) was significantly higher below the fish cages (BPC in mean 8 mgC g^{-1} at COMBI and CTRL1, Table 1) compared to the external control station CTRL2 (2.7 ± 0.2 mgC g^{-1}; PERMANOVA pairwise p < 0.05, Table S2). In the bioremediation treated sediments (COMBI stations), each biochemical component of the organic matter and BPC displayed a similar trend (Table 1), with a significant reduction of their concentration during the experimental period (BPC from 7.8 ± 1.1 at T30 to 3.2 ± 0.5 mgC g^{-1} at T240; PERMANOVA pairwise p < 0.01, Table S3). In the COMBI stations, the values during the bioremediation treatment were significantly lower when compared with those reported for CTRL1 (PERMANOVA pairwise p < 0.05, Table S2), while being generally comparable with external control station (Table 1).

During the entire treatment, the biochemical composition did not change between control and fish farm sediments, and no significant differences were observed in the quality of organic matter (Table S2). Among the organic polymers, proteins represented the largest fraction of the BPC (more than 50%) with values of the protein to carbohydrate ratios higher than 3 in all sampling systems (Table 1).

3.2. Extracellular enzymatic activities

Microbial extracellular enzymatic activities showed clear differences among the investigated stations (COMBI, CTRL1 and CTRL2; PERMANOVA results in Supplementary material; Table S4). Before the beginning of the bioremediation treatment, the aminopeptidase (MCA) and alkaline phosphatase (MUF-P) activities were significantly lower (PERMANOVA pairwise p < 0.05; Table S5) below the fish cages than at the external control station (from 2.1 ± 0.2 to 10.1 ± 2.6 nmol MCA $g^{-1}h^{-1}$ at COMBI and CTRL2, respectively and from 10.8 ± 1.7 to 17.2 ± 3.4 nmol MUF-P $g^{-1}h^{-1}$ at CTRL1 and CTRL2, respectively; Fig. 2). Conversely, β-Glucosidase (Glu-MUF) activity was significantly higher (PERMANOVA pairwise p < 0.05) inside the fish farm than outside (from 1.5 ± 0.3 at CTRL2 to 5.6 ± 0.8 nmol $g^{-1}h^{-1}$ at CTRL1; Fig. 2). We observed at COMBI

Table 1
Concentration of protein (PRT), carbohydrate (CHO), lipid (LIP) and biopolymeric carbon (BPC), protein contribution to BPC (PRT/BPC%) and protein to carbohydrate ratio (PRT:CHO), normalized aminopeptidase (MCA x Cell), alkaline phosphatase (MUF-P x Cell) and β–Glucosidase (Glu-MUF x Cell) enzymatic activities at the treated (COMBI) and untreated (CTRL1) stations of the fish farm and at the external control station (CTRL2) before (T0) and after 30 (T30), 120 (T120), 180 (T180) and 240 (T240) days from the start of bioremediation treatment.

	PRT mg g^{-1}			CHO mg g^{-1}			LIP mg g^{-1}		
Time	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd
T0	10.0 ± 1.8	13.2 ± 0.7	3.7 ± 0.3	4.2 ± 0.0	3.0 ± 0.6	1.2 ± 0.0	1.6 ± 0.3	1.7 ± 0.4	0.6 ± 0.0
T30	5.1 ± 0.3	10.3 ± 1.6	3.2 ± 0.8	1.5 ± 0.2	2.1 ± 0.4	1.7 ± 0.2	0.8 ± 0.0	1.2 ± 0.2	0.6 ± 0.0
T120	5.2 ± 0.1	11.6 ± 2.8	4.0 ± 0.1	0.7 ± 0.0	2.5 ± 0.3	1.1 ± 0.2	0.5 ± 0.0	1.5 ± 0.2	0.4 ± 0.1
T180	4.5 ± 1.1	11.8 ± 2.2	2.5 ± 0.6	1.5 ± 0.1	2.5 ± 0.3	0.8 ± 0.2	1.2 ± 0.2	1.5 ± 0.4	0.8 ± 0.2
T240	4.4 ± 1.2	12.7 ± 0.6	2.6 ± 0.6	1.2 ± 0.2	2.7 ± 0.3	0.8 ± 0.1	0.8 ± 0.0	1.6 ± 0.1	0.6 ± 0.1

	BPC mgC g^{-1}			PRT/BPC %			PRT:CHO		
Time	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd
T0	7.8 ± 1.1	8.9 ± 0.2	2.7 ± 0.2	62.6 ± 2.6	60.1 ± 0.6	72.7 ± 3.5	2.4 ± 0.5	4.6 ± 1.1	3.2 ± 0.2
T30	3.7 ± 0.1	6.8 ± 0.8	2.7 ± 0.5	67.6 ± 2.7	73.9 ± 3.1	57.4 ± 5.2	3.5 ± 0.6	5.0 ± 0.2	1.8 ± 0.3
T120	3.2 ± 0.0	7.8 ± 1.7	2.7 ± 0.1	79.5 ± 1.4	72.4 ± 2.0	72.0 ± 3.7	7.6 ± 0.2	4.6 ± 0.5	3.7 ± 0.7
T180	3.7 ± 0.7	7.9 ± 1.5	2.1 ± 0.5	58.5 ± 3.1	72.8 ± 0.3	57.6 ± 2.3	3.0 ± 0.7	4.6 ± 0.3	3.4 ± 1.3
T240	3.2 ± 0.5	8.5 ± 0.5	2.0 ± 0.4	66.3 ± 8.1	73.4 ± 1.0	62.1 ± 3.7	4.3 ± 1.8	4.8 ± 0.4	3.2 ± 1.0

	MCA x Cell nmol x 10^{-8} cell^{-1}h^{-1}			MUF-P x Cell nmol x 10^{-8} cell^{-1}h^{-1}			Glu-MUF x Cell nmol x 10^{-8} cell^{-1}h^{-1}		
Time	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd	COMBI avg sd	CTRL1 avg sd	CTRL2 avg sd
T0	0.5 ± 0.1	0.9 ± 0.2	5.2 ± 0.3	3.2 ± 0.5	2.7 ± 0.5	9.0 ± 1.5	0.6 ± 0.1	1.4 ± 0.2	0.8 ± 0.1
T30	1.0 ± 0.6	2.0 ± 0.4	8.8 ± 1.3	1.9 ± 0.1	5.4 ± 0.5	11.3 ± 1.0	0.6 ± 0.3	2.1 ± 0.1	0.6 ± 0.1
T120	1.9 ± 0.2	1.0 ± 0.2	15.4 ± 3.4	7.4 ± 1.0	3.0 ± 0.7	17.4 ± 0.5	1.0 ± 0.3	1.7 ± 0.1	1.2 ± 0.1
T180	2.1 ± 0.3	0.9 ± 0.1	8.7 ± 1.6	9.6 ± 2.6	3.0 ± 0.7	17.8 ± 3.9	1.7 ± 0.3	1.8 ± 0.5	1.2 ± 0.2
T240	4.2 ± 1.0	0.5 ± 0.1	8.6 ± 1.2	11.0 ± 2.3	1.9 ± 0.2	21.7 ± 1.4	2.6 ± 0.0	1.7 ± 0.0	1.9 ± 0.1

stations a treatment effect on MCA and MUF-P activities (Fig. 2), which showed a significant increase (PERMANOVA pairwise p < 0.01; Table S6) reaching values of 6.6 ± 1.5 nmol g^{-1}h^{-1} and 17.1 ± 3.4 nmol g^{-1}h^{-1}, respectively, at the end of the bioremediation, comparable to CTRL2 and significantly higher (PERMANOVA pairwise p < 0.01; Table S5) than at CTRL1. Glu-MUF activity (Fig. 2) at treated stations showed a significant increase only at the end of the bioremediation (from 2.4 ± 0.1 at T0 to 4.0 ± 0.01 nmol g^{-1}h^{-1} at T240; PERMANOVA pairwise p < 0.05; Table S6). However in the untreated sediment below the cages, the values were significantly higher than at the other stations (COMBI and CTRL2) (PERMANOVA pairwise p < 0.05; Table S5).

When the rates of enzymatic activities were normalized to prokaryotic abundance (Table 1), they showed consistent patterns to those displayed by the non-normalized rates, as evidenced by the significant differences observed between stations and experimental times (PERMANOVA, Tables S5 and S6).

3.3. Prokaryotic abundance and biomass

Prokaryotic abundance and biomass before the treatment (T0) were significantly higher (PERMANOVA pairwise p < 0.01; Table S5) below the fish cages (COMBI and CTRL1) compared to the external control station (CTRL2; Fig. 3a). The comparison between treated (COMBI) and control samples (CTRL1 and CTRL2) revealed that, during the experimental time, the treated samples showed a rapid and significant (PERMANOVA pairwise p < 0.05, Table S6) decrease in prokaryotic variables (total abundance and biomass) from T0 to T120 and 240 (by about 34% and 60%, respectively), reaching values comparable with the external control site (CTRL2; Fig. 3a). Prokaryotic abundance showed values on average higher at CTRL1 than at CTRL2 (3.22 ± 0.18 and 1.63 ± 0.08 cell g^{-1}, respectively; PERMANOVA pairwise p < 0.05, Table S5) during the experimental

time.

3.4. Prokaryotic richness and community composition

The results of prokaryotic diversity analyses, based on 16S rRNA gene sequencing, are summarized in Fig. 3b (richness) and Fig. 4 (community composition, at the phylum level). At the control sediments outside the fish farm (CTRL2), the richness was significantly higher than at the aquaculture sediments (COMBI and CTRL1) before the treatment (from 1095 ± 220 to 2366 ± 110 at CTRL1 and CTRL2 respectively; PERMANOVA pairwise p < 0.001; Table S5) and decreased over time (down to 1636 ± 72 at T240; PERMANOVA pairwise p < 0.01; Table S6). In the bioremediation treated samples (COMBI), prokaryotic richness showed values significantly lower at T240 than at T0 (1632 ± 18 at T0 and 1219 ± 87 at T240; PERMANOVA pairwise p < 0.001; Table S6), however this trend was not observed in the non-bioremediated aquaculture samples (CTRL1).

Results of prokaryotic community composition (Fig. 4) showed the dominance, at the phylum level, of Proteobacteria in all sediments (range 51.8−63.1% at COMBI, 38.7−66.7% at CTRL1 and 52.7−56.5% at CTRL2). Among the classes within this phylum, Epsilonproteobacteria dominated in farm sediments (on average 32.2% at COMBI and 28.3% at CTLR1; Supplementary Fig. S1) and not at the external control station (on average 2.5% at CTRL2) where, conversely, sediments appeared to be dominated by Gammaproteobacteria (on average 22.9% at CTRL2, as opposed to 5.9% at COMBI and 7.4% at CTRL1). Aquaculture sediments, unlike the control sediments outside the farm, were characterized by the higher relative abundance of Bacteroidetes (range 13.6−18.9% at COMBI, and 10.5−22.9% at CTRL1), values that were on average 1.7 and 1.6 times higher than at CTRL2 (range 8.6−11.3%; Fig. 4). A similar trend of higher abundance at the aquaculture sites was
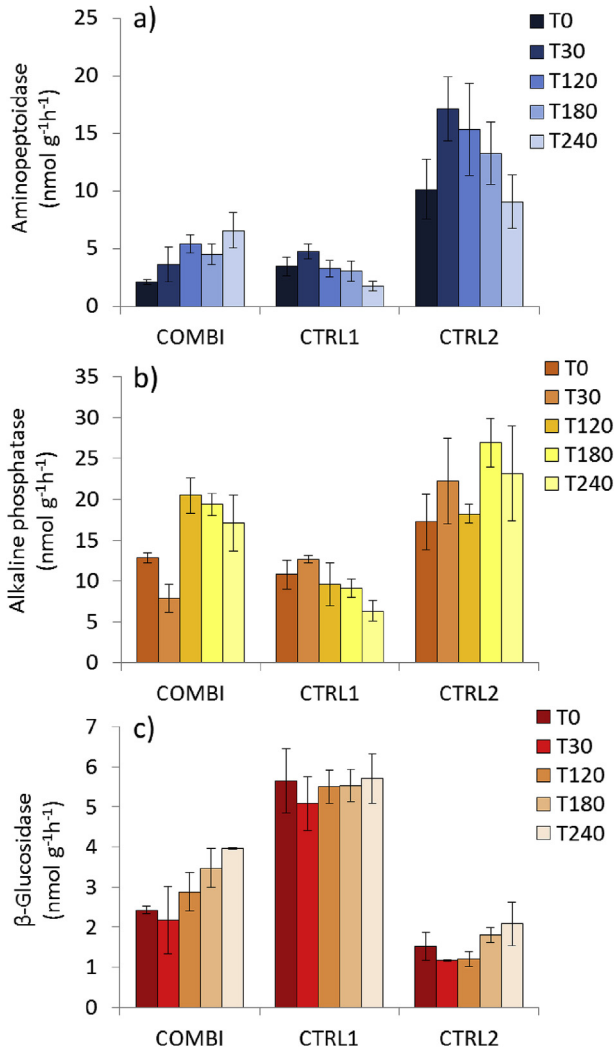
Fig. 2. Aminopeptidase (a), alkaline phosphatase (b) and β-Glucosidase (c) rates (means ± standard deviation) in the sediments at the treated (COMBI) and untreated (CTRL1) stations in the fish farm, and at the external control station (CTRL2) before (T0) and after 30 (T30), 120 (T120), 180 (T180) and 240 (T240) days from the start of bioremediation treatment.

Fig. 4. Bacterial community composition (expressed as relative abundance, %) at the phylum level in the sediment at a) the treated (COMBI) and b) untreated (CTRL1) stations in the fish farm, and at c) the external control station (CTRL2) before (T0) and after 30 (T30), 120 (T120), 180 (T180) and 240 (T240) days from the start of bioremediation treatment. "Others" includes members affiliated to all those phyla that typically accounted for <1% of the relative abundance within the assemblages.

observed for the members within the phylum Firmicutes, which were much higher at COMBI and CTRL1 that at CTRL2 (on average 10.4 and 14.9 times higher, respectively), Spirochaetes (on average 2.3 and 2.1 times higher at COMBI and CTRL1 than CTRL2) and OD1 (on average 3.4 and 1.8 times higher at COMBI and CTRL1 than at
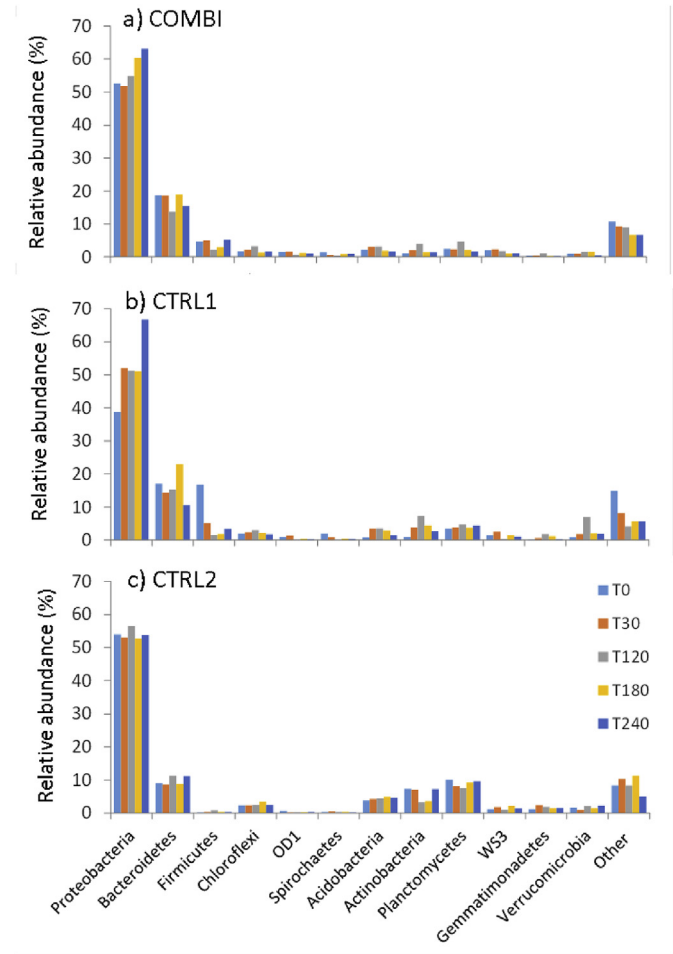
CTRL2; Fig. 4). On the other side, external control sediments showed larger relative abundance of members of the phyla Acidobacteria (on average 4.4% at CTRL2 vs. 2.3% at COMBI and 2.4% at CTRL1), Actinobacteria (on average, 5.7% at CTRL2 vs. 1.9% at COMBI and 3.8% at CTRL1) and Planctomycetes (on average 9.1% at CTRL2 vs. 2.6% at COMBI and 4% at CTRL1; Fig. 4). When comparing COMBI and CTRL1 sediments to investigate the consequences of the
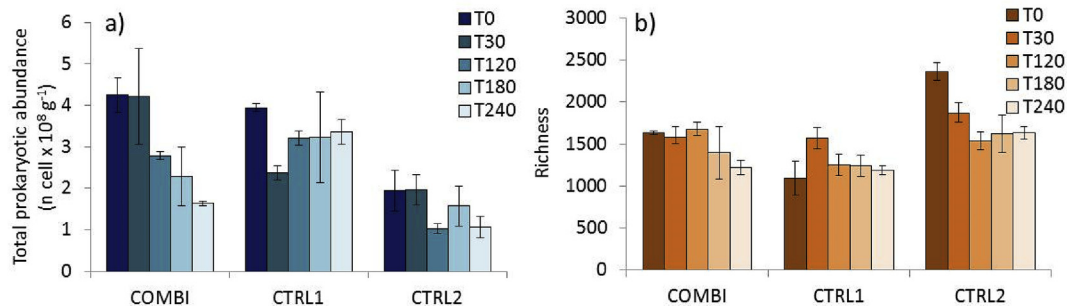

Fig. 3. Total prokaryotic abundance (a) and richness (b) (means ± standard deviation) in the sediments at the treated (COMBI) and untreated (CTRL1) stations in the fish farm, and at the external control station (CTRL2) before (T0) and after 30 (T30), 120 (T120), 180 (T180) and 240 (T240) days from the start of bioremediation treatment.

treatment, the most striking differences at the phylum level were evident in terms of Verrucomicrobia, Gemmatimonadates and Actinobacteria (on average two-times lower at COMBI that at CTRL1), while members of the OD1 were ca. double at COMBI than at CTRL1.

Linear Discriminant Analysis Effect Size (LEfSe) with Linear Discriminant Analysis (LDA) was used to identify bacterial taxa showing statistically significant differences between treated (COMBI) and non-treated samples inside (CTRL1) and outside (CTRL2) the fish farm (Fig. 5). A number of 29 taxa (at the family taxonomic level) were distinguishable between the treatments. When comparing the COMBI with the CTRL1 sediments, in order to test the effects of the bioremediation treatment in the aquaculture sediments more efficiently, we observed that the CTRL1 sediments were significantly enriched in certain taxa (Fig. 6), including several anaerobic bacteria (e.g., Clostridiales, Acidaminobacteraceae). Conversely, COMBI sediments showed a significantly higher relative abundance of Lachnospyraceae, Campylobacteraceae and Marinilabiaceae (Fig. 6).

Fig. 5. Results of LEfSe analyses on the 16S rRNA sequences. a) Histogram of the LDA scores computed for taxa that are differentially abundant between treatments (COMBI, CTRL1 and CTRL2), which provides the effect sizes of the observed differences. b) Taxonomic cladogram based on LDA which classifies discriminative taxonomic differences between the treatments. Moving from inside to outside, the five rings of the cladogram stand for the phylum, class, order and family taxonomic level. The taxa with non-significant differences are represented as brown/light green symbols. The diameter of the symbols is proportional to the relative abundance.

4. Discussion

4.1. Aquaculture impact on the sediment trophic status and microbes

Fish farming is known to modify the organic and inorganic nutrient loading of coastal areas with a consequent negative impact on the benthic environment related to the increased spread of organic wastes (uneaten food, faecal matter and dead fishes). This effect is particularly evident in shallow and sheltered areas where hydrodynamic circulation is limited, such as gulf and semi-enclosed bays (Karakassis et al., 2000; Dell'Anno et al., 2002; Fabiano et al., 2003; Holmer et al., 2003; Vezzulli et al., 2008; Neofitou et al., 2010). The study of the biochemical composition of sedimentary organic matter can provide important information on the trophic status of the benthic system (Dell'Anno et al., 2002) and on the environmental impact of the aquaculture activity.

In this study we observed that the concentration of biopolymeric carbon in the sediments of the fish farm, located within a harbour, was higher compared to that found in other aquaculture studies (Vezzulli et al., 2004; Mirto et al., 2012). The values of each biochemical components were three to four times higher beneath the fish cages than at the control sediments, indicating a condition of organic enrichment due to fish farming (La Rosa et al., 2001). Particularly the high accumulation of protein in fish farm sediments, observed in the present study, is likely to be related to the composition of the food pellets provided to reared fish which remain, for a large fraction, typically uneaten (Vezzulli et al., 2002; Mirto et al., 2012).

The high organic matter inputs from the fish farms are generally known to promote prokaryotic degradation activity in the aquaculture area (Vezzulli et al., 2002, 2004; Caruso et al., 2003; Sakami et al., 2005; Luna et al., 2013). However, in intensive aquaculture systems, the strong accumulation of organic detritus frequently exceeds the microbial degradation capacity of the system (Meyer-Reil and Kostel, 2000; Holmer et al., 2003; Sakami et al., 2005), and a substantial fraction of the organic carbon deposited on the sediment under fish cages can be buried (McGhie et al., 2000). Consistently with this, we observed lower aminopeptidase and alkaline phosphatase activities in the fish farm sediment than in the reference site. The low microbial metabolism associated with the high organic matter inputs from fish farm can be explained by the inability of microbial activity to keep up with the organic matter loading in the sediments, reaching a saturation level (Meyer-Reil and Kostel, 2000; Holmer et al., 2003; Sakami et al., 2005). Moreover, certain biochemical components of organic matter are known to be highly oxygen reactive compounds, consequently their strong accumulation can determine a modification of the physical and chemical characteristics of the sediment and overlying water with a progressive decrease in oxygen availability for the benthos beneath the fish cages (Vezzulli et al., 2002; Holmer and Frederiksen, 2007). The depletion of oxygen, often observed in fish farm areas, could decrease prokaryotic degradation activity rates in the sediment, in particular of proteolytic enzymes (Patel et al., 2001; Sakami et al., 2005; Reichardt et al., 2011). Although we did not measure directly the Eh in the sediments, we found black sediment beneath the fish cages, which suggests the presence of highly reducing conditions also in the more surficial sediment layers.

Several studies have shown that the degradation activities toward different organic substrates in the sediment often do not follow the same patterns (Fabiano et al., 2003; Sakami et al., 2005; Caruso, 2014). According to this, we observed that β-Glucosidase activity displayed higher rates inside the fish farm than outside, showing an opposite trend respect to the other enzymatic activities. Consistently with our results, it was observed that a variety of
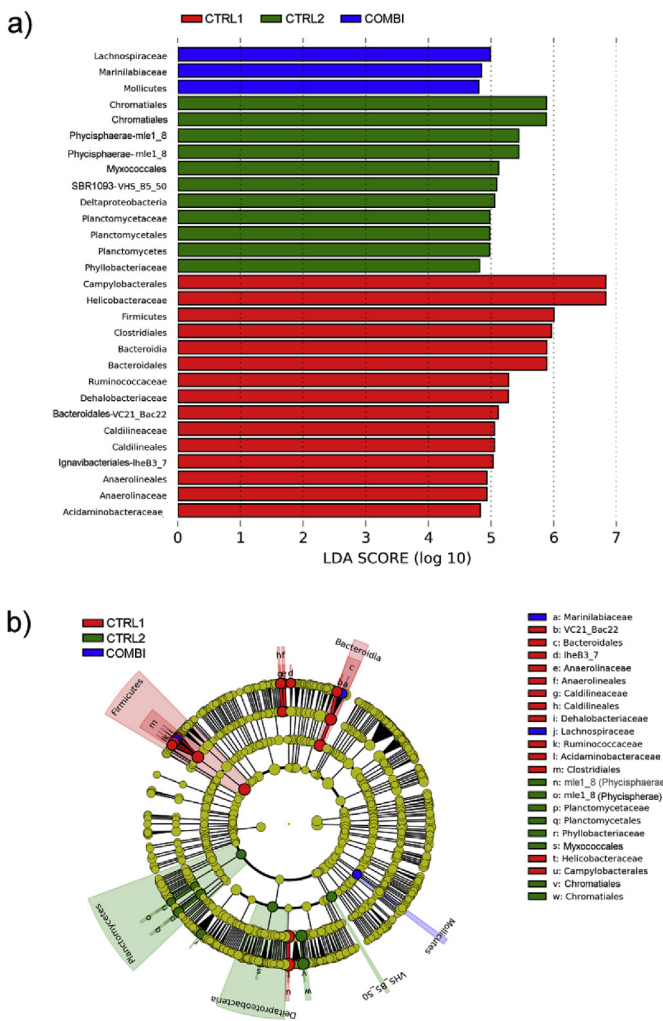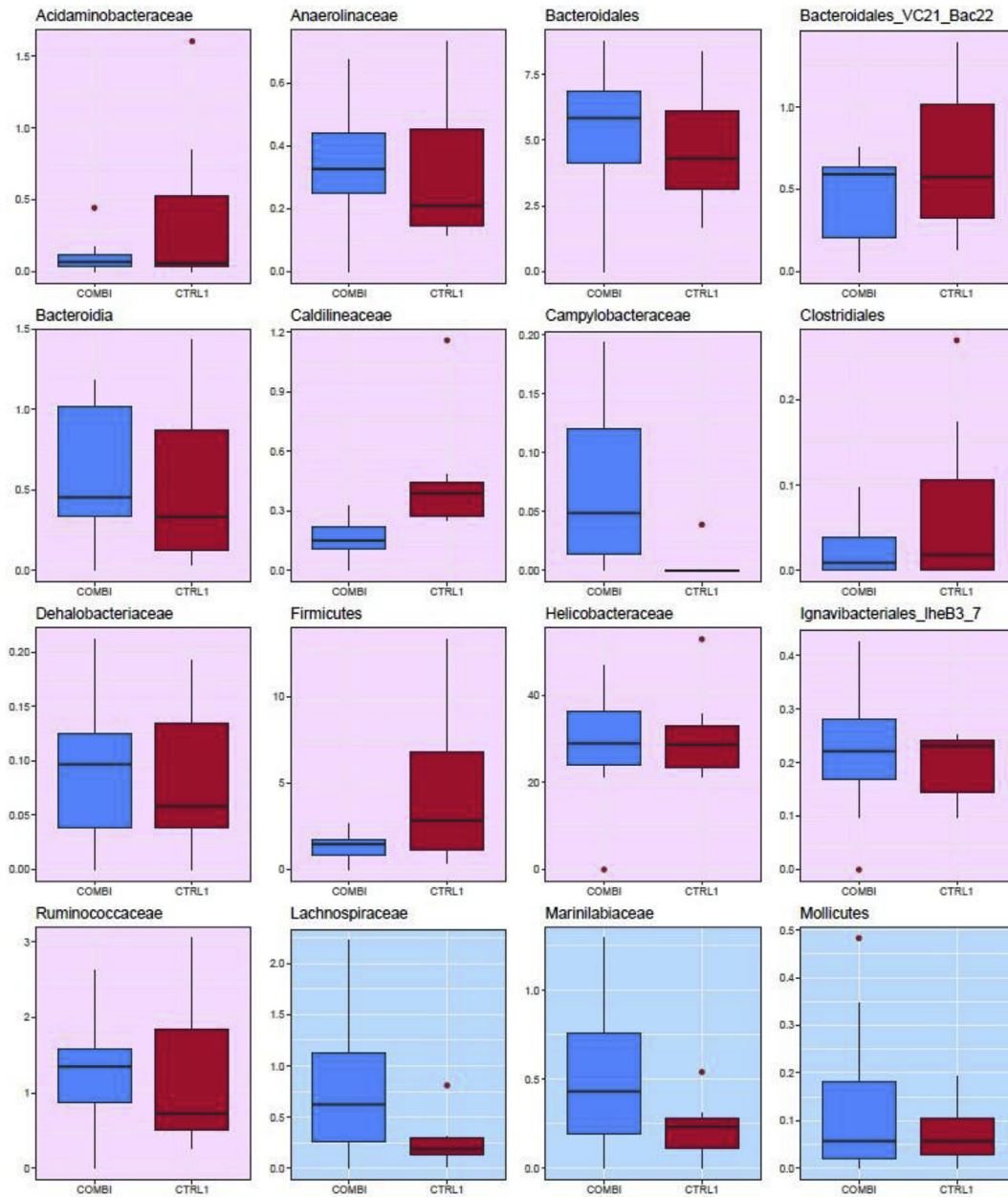
Fig. 6. Comparison in the relative abundance of the significant taxa identified by LEfSe between treated (COMBI) and untreated (CTRL1) sediments in the fish farm during the course of the bioremediation experiment.

organic components derived from fish food or the faeces of the cultured fish can promote β-Glucosidase activity but have no effect on, or even suppress, aminopeptidase activity (Sakami et al., 2005; Caruso, 2014). The low rates in organic matter degradation, observed in this study, can cause a further organic matter enrichment of the sediments, with a consequent oxygen reduction and release of toxic products that negatively affect benthic communities and farmed species (Holmer et al., 2003; Robinson et al., 2016).

The sensitivity of the benthic microbes to even minor changes in environmental conditions and biogeochemical processes might prove useful for providing information on the effects of fish farm biodeposition (Vezzulli et al., 2002; La Rosa et al., 2001; Caruso et al., 2003). According to previous studies (La Rosa et al., 2001; Vezzulli et al., 2002; Luna et al., 2013), a significant higher abundance and biomass of prokaryotes in the sediments under the fish farm cages with respect to the sediment outside the fish farm was observed, a finding generally related with the increased availability of organic matter that promotes prokaryotic growth. However, the low enzymatic microbial activities observed in the sediment beneath the fish farm cages could indicate that a large fraction of the prokaryotic assemblages is dead or dormant (Luna et al., 2002). In fact the enzymatic activities normalized to pro-karyotic cell number were low, indicating a functional stress occurring within the benthic bacterial population beneath the fish cages (Vezzulli et al., 2002, 2004).

As far as the prokaryotic diversity is concerned, prokaryotic richness was found to be lower in aquaculture sites with respect to the natural, non-impacted sediments, as expected from previous studies (Luna et al., 2013; Bissett et al., 2006), and suggesting that

the organic enrichment leads to a decrease in the number of prokaryotic species. Recent studies in salmon fish farms also reported a significantly lower bacterial diversity (measured as the inverse Simpson index) as well as a different community composition at the aquaculture compared with reference sites (Hornick and Buschmann, 2018). This shift in community composition in farm sediments was also observed in the present study, as both types of aquaculture sediments displayed higher abundance of Bacteroidetes than non-aquaculture sediments. Bacteroidetes, typically associated with high organic matter content, have been shown to be adapted to grow attached to particles, and to have great capacity to degrade high molecular weight polymers, by producing a large suite of enzymes (peptidases, glycoside hydrolases, glycosyl transferases and adhesion proteins) (Fernández-Gómez et al., 2013). Therefore, the increased relevance of Bacteroidetes in aquaculture sediments can be due to the increased availability of organic resources related to farming activities, suggesting that bacterial groups within Bacteroidetes are involved in the organic matter degradation under the fish farm. At the same time, aquaculture sediments displayed higher abundance of members within the candidate phylum OD1, previously identified in a broad range of anoxic environments (Nelson and Stegen, 2015). This finding further corroborates the likely increase in anaerobicity caused by aquaculture operations.

4.2. Effect of bioremediation on trophic and microbial variables in aquaculture sediments

In the recent years, an increasing need of biotechnological tools and strategies to face the problem of pollution (e.g., the release of waste in aquaculture) in marine environments, has emerged (Vezzulli et al., 2004). Several bioremediation studies have been carried out to assess the recovery of impacted ecosystems (Suzuki et al., 2002; Vezzulli et al., 2002, 2004; Fabiano et al., 2003; Robinson et al., 2016), however no comprehensive studies have tested in natural environment the effects and consequences of bioremediation strategies on different key sedimentary variables (trophic, microbial diversity and metabolism) within aquaculture settings. Our results indicated that, in fish-farm bioremediated sediments, the treatment determined a significant decrease of organic matter concentrations, as evidenced for all the biochemical components investigated during the experimental period. This result confirms previous findings in bioremediation studies carried out at different locations in the Mediterranean Sea (Fabiano et al., 2003; Vezzulli et al., 2004), and highlights the usefulness and efficacy of biostimulation approaches to mitigate the environmental impact of fish farming. The observed decrease in organic matter concentration in remediated farm sediments is likely dependent upon the increase in microbial degradation rates. Previous *in situ* experiments have shown that bioremediation treatments can stimulate natural pelagic and benthic processes (Fabiano et al., 2003; Gallizia et al., 2004; Vezzulli et al., 2004; Wada et al., 2005; d'Errico et al., 2013). A bacterial-enzymatic mixture, used in this study, is expected to optimize the hydrolitic phase (hydrolysis of polysaccharides to simple carbohydrates, of proteins to peptides and aminoacids, and fats to glycerol and fatty acids), thus improving and accelerating the natural biodegradation capability of sediment organic matter by the benthic microbial communities. The results showed that the microbial metabolic response to the bioremediation treatment was not immediate, but became significant at the end of the treatment or after 120 days from the start of the treatment (as seen for alkaline phosphatase), in accordance with other studies in different fish farms (Fabiano et al., 2003; Gallizia et al., 2004; Vezzulli et al., 2004). In marine environment

microbial extracellular enzymatic activity is known to be a primary step in the degradation of organic matter (Manini et al., 2003). Studies of metabolic activity of prokaryotes can provide information on the potential organic matter flow through the microbial loop, so preventing its accumulation in natural environments (Manini et al., 2003; Vezzulli et al., 2004; Caruso, 2014). The results of the present study showed that bioremediation is an effective strategy to stimulate the consuming and recycling of the organic matter in fish farm sediments, and suggested that sediment biostimulation interventions should last for months in order to be fully efficacious.

The decrease in organic matter availability in bioremediated sediments appeared to cause a decrease of prokaryotic abundance throughout the experiment, as opposed to what observed in the non-bioremediated farm sediments. However, the increase of normalized enzymatic activities suggested that the remediation treatment stimulated microbial activity, resulting in a more metabolically active microbial community at the end of the experiment.

The bioremediation experiment also affected benthic prokaryotic diversity. Despite no differences were observed in prokaryotic species richness between remediated and non-remediated farm sediments, the bioremediation strategy caused significant changes in the relative abundance of certain taxa. The decreased importance of certain anaerobic taxa in the COMBI sediments evidenced a mitigation of the consequences of the organic matter enrichment. The analysis of differentially abundant taxa by LEfSe between treated and untreated farm sediments indicated that remediated sediments displayed a decreased importance of Clostridiales, Acidaminobacteraceae and Caldilinaceae. Members of these taxa, particularly bacterial groups belonging to the class Clostridia, are assumed to be important during the anaerobic degradation of organic material in aquatic systems (Schwarz et al., 2008), and have been previously reported to be major contributors of the differences in bacterial communities between mariculture and non-mariculture sediments (Li et al., 2013). Therefore, their decrease throughout the bioremediation treatment provides an indirect indication of a decreased relevance of anaerobic metabolism in these sediments, and suggests a favourable condition for an aerobic metabolism, which is typical of non-impacted sediments.

However, during the bioremediation treatment, a larger importance of members of Lachnospiraceae was observed in bioremediated sediments as compared to the non-treated ones, suggesting that the bioactivator had no effect on the removal of faecal bacteria from farmed fishes. In fact, these bacteria are among the dominant members of the gut microbiota of sea bream (Parma et al., 2016) and of several other fishes (Larsen et al., 2014; Miyake et al., 2015).

5. Conclusions

This study investigated the efficacy of a bioremediation strategy to improve the quality of organically enriched fish farm sediments by the biostimulation of in *situ* microbial degradation processes. The results indicated that the bioactivator was effective in increasing the microbial enzymatic degradation rates of organic matter, in mitigating the organic enrichment in the fish farm sediments, and in promoting a shift from an anaerobic to an aerobic metabolism in the prokaryotic community. At the same time, the treatment demonstrated to be not particularly efficient in mitigating the accumulation of faecal bacteria originating from the farmed fishes over time. Among the consequences of aquaculture, the large and constant release of fish faeces and of gut-associated microbes secreted into the faeces into the sea floor, in addition to the high organic matter load from the uneaten feed, is causing increasing environmental and sanitary concerns (Reid et al., 2009),

also in light of fish-associated microbes determining the spread of antibiotic resistance genes into the environment (Muziasari et al., 2017). Future research should focus on developing improved bio-activators that are also able to bioremediate the sediment from the fish-faeces related microbes, for instance by promoting the growth of certain aerobic microbes able to outcompete Lachnospiraceae and other gut-related microbes, in order to identify more successful bioremediation intervention of fish farm sediments.

Conflicts of interest

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest.

Three of the authors are employed by the company producing the tested bioactivator (EUROVIX S.p.A.).

Nevertheless, the research did not received financial support from the EUROVIX, and the authors employed in the company, did not influence the research outcomes. They provided only logistical support and indications on the mode of use of bioactivator (i.e., distribution, application time, dosage).

Author contributions

Francesca Ape: Conceptualization, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. **Elena Manini**: Formal analysis, Writing - original draft, Writing - review & editing. **Grazia Marina Quero**: Formal analysis, Writing - review & editing. **Gian Marco Luna**: Formal analysis, Resources, Writing - original draft, Writing - review & editing. **Gianluca Sarà**; : Resources, Supervision. **Paolo Vecchio**: Conceptualization, Supervision. **Pierlorenzo Brignoli**: Conceptualization, Supervision. **Simone Mirto**: Conceptualization, Resources, Writing - review & editing, Supervision, Project administration, Funding acquisition

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.03.178.

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46. https://doi.org/10.1111/j.1442-9993.2001.tb00081.x.

Andreolli, M., Lampis, S., Brignoli, P., Vallini, G., 2015. Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. J. Environ. Manag. 153, 121–131. https://doi.org/10.1016/j.jenvman.2015.02.007.

Bissett, A., Bowman, J., Burke, C., 2006. Bacterial diversity in organically-enriched fish farm sediments. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 55 (1), 48–56. https://doi.org/10.1111/j.1574-6941.2005.00012.x.

Bouwman, A.F., Beusen, A.H.W., Overbeek, C.C., Bureau, D.P., Pawlowski, M., Glibert, P.M., 2013. Hindcasts and future projections of global inland and coastal nitrogen and phosphorus loads due to finfish aquaculture. Rev. Fish. Sci. 21 (2), 112–156. https://doi.org/10.1080/10641262.2013.790340.

Casado-Coy, N., Martínez-García, E., Sánchez-Jerez, P., Sanz-Lázaro, C., 2017. Mollusc-shell debris can mitigate the deleterious effects of organic pollution on marine sediments. J. Appl. Ecol. 54 (2), 547–556. https://doi.org/10.1111/1365-2664.12748.

Caruso, G., 2014. Effects of aquaculture activities on microbial assemblages. Oceanography 02 (02). https://doi.org/10.4172/2332-2632.1000e107.

Caruso, G., Genovese, L., Mancuso, M., Modica, A., 2003. Effects of fish farming on microbial enzyme activities and densities: comparison between three Mediterranean sites. Lett. Appl. Microbiol. 37 (4), 324–328. https://doi.org/10.1046/j.1472-765X.2003.01401.x.

Cho, B.C., Azam, F., 1990. Biogeochemical significance of bacterial biomass in the ocean's euphotic zone. Mar. Ecol. Prog. Ser. 63, 253–259. https://doi.org/10.3354/meps063253.

Danovaro, R., Manini, E., Fabiano, M., 2002. Exo-enzymatic activity and organic matter composition in sediments of the Northern Adriatic Sea: response to a river plume. Microb. Ecol. 44 (3), 235–251. https://doi.org/10.1007/s00248-002-1023-2.

Dell'Anno, A., Mei, M.L., Pusceddu, A., Danovaro, R., 2002. Assessing the trophic state and eutrophication of coastal marine systems: a new approach based on the biochemical composition of sediment organic matter. Mar. Pollut. Bull. 44 (7), 611–622. https://doi.org/10.1016/S0025-326X(01)00302-2.

Dell'Anno, A., Beolchini, F., Gabellini, M., Rocchetti, L., Pusceddu, A., Danovaro, R., 2009. Bioremediation of petroleum hydrocarbons in anoxic marine sediments: consequences on the speciation of heavy metals. Mar. Pollut. Bull. 58 (12), 1808–1814. https://doi.org/10.1016/j.marpolbul.2009.08.002.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K., Huber, T., Dalevi, D., Hu, P., Andersen, G.L., 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl. Environ. Microbiol. 72 (7), 5069–5072. https://doi.org/10.1128/AEM.03006-05.

D'Errico, G., Giovannelli, D., Montano, C., Milanovic, V., Ciani, M., Manini, E., 2013. Bioremediation of high organic load lagoon sediments: compost addition and priming effects. Chemosphere 91 (1), 99–104. https://doi.org/10.1016/j.chemosphere.2012.11.037.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Eiler, A., Heinrich, F., Bertilsson, S., 2012. Coherent dynamics and association networks among lake bacterioplankton taxa. ISME J. 6 (2), 330–342. https://doi.org/10.1038/ismej.2011.113.

Fabiano, M., Danovaro, R., 1994. Composition of organic matter in sediments facing a river estuary (Tyrrhenian Sea): relationships with bacteria and microphytobenthic biomass. Hydrobiologia 277, 71–84.

Fabiano, M., Marrale, D., Misic, C., 2003. Bacteria and organic matter dynamics during a bioremediation treatment of organic-rich harbour sediments. Mar. Pollut. Bull. 46 (9), 1164–1173. https://doi.org/10.1016/S0025-326X(03)00166-8.

FAO (Food and Agriculture Organization of the United Nations), 2008. Glossary of Aquaculture. FAO Fisheries and Aquaculture, Rome.

FAO (Food and Agriculture Organization of the United Nations), 2018. The State of World Fisheries and Aquaculture. FAO, Rome.

Fernández-Gómez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S.G., González, J.M., Pedrós-Alió, C., 2013. Ecology of marine Bacteroidetes: a comparative genomics approach. ISME J. 7 (5), 1026–1037. https://doi.org/10.1038/ismej.2012.169.

Gallizia, I., Vezzulli, L., Fabiano, M., 2004. Oxygen supply for biostimulation of enzymatic activity in organic-rich marine ecosystems. Soil Biol. Biochem. 36 (10), 1645–1652. https://doi.org/10.1016/j.soilbio.2004.07.007.

Genovese, M., Crisafi, F., Denaro, R., Cappello, S., Russo, D., Calogero, R., Santisi, S., Catalfamo, M., Modica, A., Smedile, F., Genovese, L., Golyshin, P.N., Giuliano, L., Yakimov, M.M., 2014. Effective bioremediation strategy for rapid in situ cleanup of anoxic marine sediments in mesocosm oil spill simulation. Front. Microbiol. 5, 1–14. https://doi.org/10.3389/fmicb.2014.00162.

Guo, L., Chen, Q., Fang, F., Hu, Z., Wu, J., Miao, A., Xiao, L., Chen, X., Yang, L., 2013. Application potential of a newly isolated indigenous aerobic denitrifier for nitrate and ammonium removal of eutrophic lake water. Bioresour. Technol. 142, 45–51. https://doi.org/10.1016/j.biortech.2013.05.021.

Holmer, M., Frederiksen, M.S., 2007. Stimulation of sulfate reduction rates in Mediterranean fish farm sediments inhabited by the seagrass Posidonia oceanica. Biogeochemistry 85 (2), 169–184. https://doi.org/10.1007/s10533-007-9127-x.

Holmer, M., Perez, M., Duarte, C.M., 2003. Benthic primary producers: a neglected environmental problem in Mediterranean maricultures? Marine Pollution Bullettin 46, 1372–1376. https://doi.org/10.1016/S0025-326X(03)00396-5.

Hoppe, H.G., 1993. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) of bacteria. In: Kemp, P.F., Sherr, B.F., Sherr, E.B., Cole, J.J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, FL, USA, pp. 423–431.

Hornick, K.M., Buschmann, A.H., 2018. Insights into the diversity and metabolic function of bacterial communities in sediments from Chilean salmon aquaculture sites. Ann. Microbiol. 68 (2), 63–77. https://doi.org/10.1007/s13213-017-1317-8.

Huo, Y., Wu, H., Chai, Z., Xu, S., Han, F., Dong, L., He, P., 2012. Bioremediation efficiency of Gracilaria verrucosa for an integrated multi-trophic aquaculture system with Pseudosciaena crocea in Xiangshan harbor, China. Aquaculture 326–329, 99–105. https://doi.org/10.1016/j.aquaculture.2011.11.002.

Karakassis, I., Tsapakis, M., Hatziyanni, E., Papadopoulou, K.N., Plaiti, W., 2000. Impact of cage farming of fish on the seabed in three Mediterranean coastal areas. ICES (Int. Counc. Explor. Sea) J. Mar. Sci. 57 (5), 1462–1471. https://doi.org/10.1006/jmsc.2000.0925.

Klaoudatos, S.D., Klaoudatos, D.S., Smith, J., Bogdanos, K., Papageorgiou, E., 2006. Assessment of site specific benthic impact of floating cage farming in the eastern Hios island, Eastern Aegean Sea, Greece. J. Exp. Mar. Biol. Ecol. 338 (1), 96—111. https://doi.org/10.1016/j.jembe.2006.07.002.

Kunihiro, T., Takasu, H., Miyazaki, T., Uramoto, Y., Kinoshita, K., Yodnarasri, S., Hama, D., Wada, M., Kogure, K., Ohwada, K., Tsutsumi, H., 2011. Increase in Alphaproteobacteria in association with a polychaete, Capitella sp. I, in the organically enriched sediment. ISME J. 5 (11), 1818—1831. https://doi.org/10.1038/ismej.2011.57.

La Rosa, T., Mirto, S., Mazzola, A., Danovaro, R., 2001. Differential responses of benthic microbes and meiofauna to fish-farm disturbance in coastal sediments. Environ. Pollut. 112 (3), 427—434. https://doi.org/10.1016/S0269-7491(00)00141-X.

Larsen, A.M., Mohammed, H.H., Arias, C.R., 2014. Characterization of the gut microbiota of three commercially valuable warmwater fish species. J. Appl. Microbiol. 116 (6), 1396—1404. https://doi.org/10.1111/jam.12475.

Li, J., Li, F., Yu, S., Qin, S., Wang, G., 2013. Impacts of mariculture on the diversity of bacterial communities within intertidal sediments in the northeast of China. Microb. Ecol. 66 (4), 861—870. https://doi.org/10.1007/s00248-013-0272-6.

Luna, G.M., Manini, E., Danovaro, R., 2002. Large fraction of dead and inactive bacteria in coastal marine sediments: comparison of protocols for determination and ecological significance. Appl. Environ. Microbiol. 68 (7), 3509—3513. https://doi.org/10.1128/AEM.68.7.3509-3513.2002.

Luna, G.M., Corinaldesi, C., Dell'Anno, A., Pusceddu, A., Danovaro, R., 2013. Impact of aquaculture on benthic virus-prokaryote interactions in the Mediterranean Sea. Water Res. 47 (3), 1156—1168. https://doi.org/10.1016/j.watres.2012.11.036.

Manini, E., Fiordelmondo, C., Gambi, C., Pusceddu, A., Danovaro, R., 2003. Benthic microbial loop functioning in coastal lagoons: a comparative approach. Oceanol. Acta 26 (1), 27—38. https://doi.org/10.1016/S0399-1784(02)01227-6.

McArdle, B.H., Anderson, M.J., 2001. Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82 (1), 290—297. https://doi.org/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2.

McGhie, T.K., Crawford, C.M., Mitchell, I.M., O'Brien, D., 2000. The degradation of fish-cage waste in sediments during fallowing. Aquaculture 187 (3—4), 351—366. https://doi.org/10.1016/S0044-8486(00)00317-3.

Meyer-Reil, L.A., Kostel, M., 2000. Eutrophication of marine waters: effects on benthic microbial communities. Mar. Pollut. Bull. 41 (1—6), 255—263. https://doi.org/10.1016/S0025-326X(00)00114-4.

Mirto, S., Gristina, M., Sinopoli, M., Maricchiolo, G., Genovese, L., Vizzini, S., Mazzola, A., 2012. Meiofauna as an indicator for assessing the impact of fish farming at an exposed marine site. Ecol. Indicat. 18, 468—476. https://doi.org/10.1016/j.ecolind.2011.12.015.

Miyake, S., Ngugi, D.K., Stingl, U., 2015. Diet strongly influences the gut microbiota of surgeonfishes. Mol. Ecol. 24 (3), 656—672. https://doi.org/10.1111/mec.13050.

Muziasari, W.I., Pitkänen, L.K., Sørum, H., Stedtfeld, R.D., Tiedje, J.M., Virta, M., 2017. The resistome of farmed fish feces contributes to the enrichment of antibiotic resistance genes in sediments below Baltic Sea fish farms. Front. Microbiol. 7, 2137. https://doi.org/10.3389/fmicb.2016.02137.

Nelson, W.C., Stegen, J.C., 2015. The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. Front. Microbiol. 6, 1—14. https://doi.org/10.3389/fmicb.2015.00713.

Neofitou, N., Vafidis, D., Klaoudatos, S., 2010. Spatial and temporal effects of fish farming on benthic community structure in a semi-enclosed gulf of the Eastern Mediterranean. Aquaculture Environ. Interactions 1 (2), 95—105. https://doi.org/10.3354/aei00010.

Parma, L., Candela, M., Soverini, M., Turroni, S., Consolandi, C., Brigidi, P., Mandrioli, L., Sirri, R., Fontanillas, R., Gatta, P.P., Bonaldo, A., 2016. Next-generation sequencing characterization of the gut bacterial community of gilthead sea bream (Sparus aurata, L.) fed low fishmeal based diets with increasing soybean meal levels. Anim. Feed Sci. Technol. 222, 204—216. https://doi.org/10.1016/j.anifeedsci.2016.10.022.

Patel, A.B., Fukami, K., Nishijima, T., 2001. Extracellular proteolytic activity in the surface sediment of a eutrophic inlet. Microb. Environ. 16 (1), 25—35. https://doi.org/10.1264/jsme2.2001.25.

Pusceddu, A., Dell'Anno, A., Danovaro, R., Manini, E., Sara, G., Fabiano, M., 2003. Enzymatically hydrolyzable protein and carbohydrate sedimentary pools as indicators of the trophic state of detritus sink systems: a case study in a Mediterranean coastal lagoon. Estuaries 26 (3), 641—650. https://doi.org/10.1007/bf02711976.

Pusceddu, A., Bianchelli, S., Canals, M., Sanchez-Vidal, A., Durrieu De Madron, X., Heussner, S., Lykousis, V., de Stigter, H., Trincardi, F., Danovaro, R., 2010. Organic matter in sediments of canyons and open slopes of the Portuguese, Catalan, Southern Adriatic and Cretan Sea margins. Deep-Sea Res. Part I 57 (3), 441—457. https://doi.org/10.1016/j.dsr.2009.11.008.

Quero, G.M., Perini, L., Pesole, G., Manzari, C., Lionetti, C., Bastianini, M., Marini, M., Luna, G.M., 2017. Seasonal rather than spatial variability drives planktonic and benthic bacterial diversity in a microtidal lagoon and the adjacent open sea. Mol. Ecol. 26 (21), 5961—5973. https://doi.org/10.1111/mec.14363.

Reichardt, W., Bolalin, G.V., Cutchon, X.D.R., Que, F.V.J., Balgos, M.J.T.D., 2011.

Enzymatic degradation of proteinaceous compounds in sediments exposed to intensive mariculture in the tropics. Aquat. Ecosys. Health Manag. 14 (3), 318—324. https://doi.org/10.1080/14634988.2010.525199.

Reid, G.K., Liutkus, M., Robinson, S.M.C., Chopin, T.R., Blair, T., Lander, T., Mullen, J., Page, F., Moccia, R.D., 2009. A review of the biophysical properties of salmonid faeces: implications for aquaculture waste dispersal models and integrated multi-trophic aquaculture. Aquacult. Res. 40 (3), 257—273. https://doi.org/10.1111/j.1365-2109.2008.02065.x.

Reimers, C.E., Alleau, Y., Bauer, J.E., Delaney, J., Girguis, P.R., Schrader, P.S., Stecher, H.A., 2013. Redox effects on the microbial degradation of refractory organic matter in marine sediments. Geochem. Cosmochim. Acta 121, 582—598. https://doi.org/10.1016/j.gca.2013.08.004.

Robinson, G., Caldwell, G.S., Wade, M.J., Free, A., Jones, C.L.W., Stead, S.M., 2016. Profiling bacterial communities associated with sediment-based aquaculture bioremediation systems under contrasting redox regimes. Sci. Rep. 6, 1—13. https://doi.org/10.1038/srep38850.

Rosa, R., Marques, A., Nunes, M.L., 2012. Impact of climate change in Mediterranean aquaculture. Rev. Aquacult. 4 (3), 163—177. https://doi.org/10.1111/j.1753-5131.2012.01071.x.

Sakami, T., Yokoyama, H., Ishihi, Y., 2005. Microbial hydrolytic enzyme activity in the sediments of a net cage aquaculture area. Fish. Sci. 71 (2), 271—278. https://doi.org/10.1111/j.1444-2906.2005.00960.x.

Sanderson, J.C., Dring, M.J., Davidson, K., Kelly, M.S., 2012. Culture, yield and bioremediation potential of Palmaria palmata (Linnaeus) Weber & Mohr and Saccharina latissima (Linnaeus) C.E. Lane, C. Mayes, Druehl & G.W. Saunders adjacent to fish farm cages in northwest Scotland. Aquaculture 354— 355, 128—135. https://doi.org/10.1016/j.aquaculture.2012.03.019.

Schwarz, J.I., Eckert, W., Conrad, R., 2008. Response of the methanogenic microbial community of a profundal lake sediment (Lake Kinneret, Israel) to algal deposition. Limnol. Oceanogr. 53 (1), 113—121. https://doi.org/10.4319/lo.2008.53.1.0113.

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome Biol. 12 (6). https://doi.org/10.1186/gb-2011-12-6-r60.

Suzuki, Y., Kelly, S.D., Kemner, K.M., Banfield, J.F., 2002. Radionuclide contamination: nanometre-size products of uranium bioreduction. Nature 419, 134. https://doi.org/10.1038/419134a.

Vezzulli, L., Chelossi, E., Riccardi, G., Fabiano, M., 2002. Bacterial community structure and activity in fish farm sediments of the Ligurian sea (Western Mediterranean). Aquacult. Int. 10 (2), 123—141. https://doi.org/10.1023/A:1021365829687.

Vezzulli, L., Pruzzo, C., Fabiano, M., 2004. Response of the bacterial community to in situ bioremediation of organic-rich sediments. Mar. Pollut. Bull. 49 (9—10), 740—751. https://doi.org/10.1016/j.marpolbul.2004.05.010.

Vezzulli, L., Moreno, M., Marin, V., Pezzati, E., Bartoli, M., Fabiano, M., 2008. Organic waste impact of capture-based Atlantic bluefin tuna aquaculture at an exposed site in the Mediterranean Sea. Estuar. Coast Shelf Sci. 78 (2), 369—384. https://doi.org/10.1016/j.ecss.2008.01.002.

Wada, M., Wu, S.S., Kogure, K., Tsutsumi, H., 2005. Short-term impact of biological activities of a burrowing polychaete, Capitella sp. I, on bacterial abundance and the chemical characteristics in organically enriched sediment. Benthos Res. 60, 59—66. https://doi.org/10.5179/benthos1996.60.2_59.

Wang, L., Li, J., Kang, W.L., 2007. Bioremediation of eutrophicated water by acinetobacter calcoaceticus. Bull. Environ. Contam. Toxicol. 78 (6), 527—530. https://doi.org/10.1007/s00128-007-9169-8.

Wu, H., Huo, Y., Hu, M., Wei, Z., He, P., 2015. Eutrophication assessment and bioremediation strategy using seaweeds co-cultured with aquatic animals in an enclosed bay in China. Mar. Pollut. Bull. 95 (1), 342—349. https://doi.org/10.1016/j.marpolbul.2015.03.016.

Yu, Z., Zhu, X., Jiang, Y., Luo, P., Hu, C., 2014. Bioremediation and fodder potentials of two Sargassum spp. in coastal waters of Shenzhen, South China. Mar. Pollut. Bull. 85 (2), 797—802. https://doi.org/10.1016/j.marpolbul.2013.11.018.

Zhang, Z., Lo, I.M.C., 2015. Biostimulation of petroleum-hydrocarbon-contaminated marine sediment with co-substrate: involved metabolic process and microbial community. Appl. Microbiol. Biotechnol. 99 (13), 5683—5696. https://doi.org/10.1007/s00253-015-6420-9.

Zhang, Z., Lo, I.M.C., Yan, D.Y.S., 2015. An integrated bioremediation process for petroleum hydrocarbons removal and odor mitigation from contaminated marine sediment. Water Res. 83, 21—30. https://doi.org/10.1016/j.watres.2015.06.022.

Zhang, H., Wang, Y., Chen, S., Zhao, Z., Feng, J., Zhang, Z., Lu, K., Jia, J., 2018. Water bacterial and fungal community compositions associated with urban lakes, Xi'an, China. Int. J. Environ. Res. Public Health 15, 469. https://doi.org/10.3390/ijerph15030469.

Zhang, H., Zhao, Z., Chen, S., Feng, J., Wang, Y., Jia, J., Li, S., Wang, Y., Yan, M., Lu, K., Hao, H., 2019. Geographical patterns of nirS gene abundance and nirS-Type denitrifying bacterial community associated with activated sludge from different wastewater treatment plants. Microb. Ecol. 77, 304—316. https://doi.org/10.1007/s00248-018-1236-7.