Marine Pollution Bulletin 180 (2022) 113759

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

The fate of conventional and potentially degradable gillnets in a seawater-sediment system

Odd G. Brakstad [a,*], Lisbet Sørensen [a], Sigrid Hakvåg [a], Heidi M. Føre [b], Biao Su [c], Marianne Aas [a], Deni Ribicic [c], Eduardo Grimaldo [d]

[a] SINTEF Ocean, Dept. Climate and Environment, Norway
[b] SINTEF Ocean, Dept. Energy and Transport, Norway
[c] SINTEF Ocean, Dept. Aquaculture, Norway
[d] SINTEF Ocean, Dept. Fisheries and New Biomarine Industry, Norway

ARTICLE INFO

Keywords:
Plastic
Gillnets
Marine environment
Ghost fishing
Fate
Degradation

ABSTRACT

Abandoned gillnets in the marine environment represent a global environmental risk due to the ghost fishing caused by the nets. Degradation of conventional nylon gillnets was compared to that of nets made of polybutylene succinate co-adipate-co-terephthalate (PBSAT) that are designed to degrade more readily in the environment. Gillnet filaments were incubated in microcosms of natural seawater (SW) and marine sediments at 20 °C over a period of 36 months. Tensile strength tests and scanning electron microscopy analyses showed weakening and degradation of the PBSAT filaments over time, while nylon filaments remained unchanged. Pyrolysis-gas chromatography/mass spectrometry revealed potential PBSAT degradation products associated with the filament surfaces, while nylon degradation products were not detected by these analyses. Microbial communities differed significantly between the biofilms on the nylon and PBSAT filaments. The slow deterioration of the PBSAT gillnet filaments shown here may be beneficial and reduce the ghost fishing periods of these gillnets.

1. Introduction

Abandoned, lost, or otherwise discarded fishing gear from commercial and recreational fishing represents a considerable global, conservational, and economic problem. According to a UN report, approximately 640,000 tons of discarded fishing nets are lost annually to the oceans worldwide (Macfadyen et al., 2009; Good et al., 2010), causing starvation, predation, cannibalism, disease, or poor water quality (Lively and Good, 2019). Ghost nets are mainly passive gears like gillnets, tangle nets, trammel nets, and traps, whereas active gears like trawls and seines are not considered to contribute much to ghost fishing (Brown et al., 2005; Gilman et al., 2016; Thomas and Sandhya, 2019). Gillnets are among the most common fishing gears in the world, and nearly 14,000 nets are estimated to be lost annually in Norway alone (Sundt et al., 2018). While gillnets were produced from degradable materials like cotton or hemp 50–60 years ago, they are now mainly composed of decay-resistant polyamide (PA; nylon), which may persist in the marine environment for many years (Thomas and Sandhya, 2019).

Attempts have been made to reduce the impacts of ghost fishing by the introduction of biodegradable materials. Biodegradable plastics such as polycaprolactone and polyhydroxyalkanoate were tested in escape rings on crab pots and did not adversely affect catches (Bilkovic et al., 2012). Recent studies have suggested that gillnets made of polybutylene succinate (PBS) and PBS resin blended with polybutylene adipate-co-terephthalate (PBAT/PBSAT) (Fig. SI1) may be used as alternatives to conventional PA gillnets to prevent ghost fishing (Kim et al., 2014a; Kim et al., 2014b; Kim et al., 2016; Kim et al., 2019). However, testing of PBSAT gillnets under commercial fishing conditions during a winter season in Northern Norway showed that cod (*Gadus morhua*) catch efficiencies were reduced by 21% for PBSAT compared to nylon gillnets, and that the PBSAT gillnets progressively lost catch efficiency over time (Grimaldo et al., 2019; Grimaldo et al., 2020). Grimaldo et al. (2019) suggested that reductions in tensile strength and elasticity of PBSAT gillnets were not only caused by use and wear, but also potentially by biological degradation. Other researchers reported that degradation of PBSAT gillnets started after 2 years in the marine environment (Kim et al., 2016; Su et al., 2019). However, microbes associated with this

* Corresponding author at: SINTEF Ocean AS, Postboks 4762, Torgarden, 7465 Trondheim, Norway.
E-mail address: odd.g.brakstad@sintef.no (O.G. Brakstad).

https://doi.org/10.1016/j.marpolbul.2022.113759
Received 17 February 2022; Received in revised form 10 May 2022; Accepted 12 May 2022
Available online 20 May 2022

degradation have not yet been identified, and the degree of degradation due to microbial activity has not been determined.

PBS and PBSAT are thermoplastic polyesters with a wide range of desirable properties, including melt processability and both thermal and chemical resistance. Aerobic biodegradation is expected to occur mainly in the aliphatic rather than the aromatic entities of the polymer, resulting in enrichment of aromatic constituents (Witt et al., 1995). PBS-degrading microorganisms are widely distributed in the environment and include actinomycetes, Proteobacteria, and fungi (Pranamuda et al., 1995; Suyama et al., 1998; Ishii et al., 2008; Tokiwa et al., 2009). The ester linkages (Fig. SI1) may be attacked by esterases and lipases in the environment (Tokiwa et al., 2009; Yamamoto-Tamura et al., 2015), and fungal hydrolytic degradation of the ester bonds has been indicated, with 10–30% mineralization during 100 days of incubation (Saadi et al., 2013). Anaerobic polyester degradation has also been reported (Pathak, 2017), but PBS degradation under anoxic conditions has not been reported. Several studies found that PBAT can be degraded by actinomycetes and fungi (Jiang et al., 2006; Kijchavengkul et al., 2010; Meyer-Cifuentes et al., 2020). However, most PBAT-degrading microorganisms cannot use the monomers (adipic acid, 1,4-butanediol and terephthalic acid) as carbon sources, suggesting that bacterial cooperation is needed for complete mineralization (Meyer-Cifuentes et al., 2020).

The extent of environmental degradation of PBS and PBAT has mainly been predicted from experiments performed in soil and compost, by respirometric analysis, or by indirect methods such as weight loss and tensile strength testing. Testing of PBS filaments in a 120 day soil burial test showed a significant decrease of tensile strength, notched Izod impact strength, and weight loss, which were interpreted as being results of soil biodegradation (Kim et al., 2005). Wang et al. (2015) reported that PBAT underwent weight reduction in a 3-month soil burial test, even in the presence of an antimicrobial substance. Laboratory tests using polyesters such as PBS and PBAT may result in a biological oxygen demand, which is a measure of ultimate biodegradation at temperatures of 22–27 °C (Nakayama et al., 2019; Yamano et al., 2019). Testing of PBAT by CO_2-evolution tests in manure, food, and yarn compost showed first-order biodegradation rates ranging from 0.0593 ± 0.0025 day^{-1} to 0.0237 ± 0.0017 day^{-1}, corresponding to mineralization half-lives of 12–30 days; however, small weight reductions indicated that side chains rather than polymer backbones were attacked during biodegradation (Kijchavengkul et al., 2010). PBSAT resins include both crystalline and amorphous structures (Kim et al., 2017), which affect biodegradation because microbial enzymes can more easily penetrate deeper into amorphous structures than more rigid and compact crystalline structures (Tokiwa et al., 2009). However, no proof of PBS/PBAT/PBSAT depolymerization in marine environments has been reported to date. Conventional gillnets composed of synthetic PA are considered to be non-biodegradable in natural environments. However, Yamano et al. (2008) showed that PAs such as nylon 4 can be degraded to monomers of γ-aminobutyric acid by isolates of *Pseudomonas* sp., and Tachibana et al. (2013) suggested that they may be biodegradable in marine environments (Tachibana et al., 2013).

Despite the lower catch efficiencies reported for degradable PBS and PBSAT gillnets, these nets may have the great advantage of reducing ghost fishing caused by abandoned, lost, or otherwise discarded fishing nets. However, the main degradation properties of these nets in the marine environment have not been properly characterized, and it remains to be determined whether degradation is caused by physical, chemical, or biological processes. Therefore, we conducted a laboratory study to compare gillnet degradation properties between conventional PA and PBAT nets incubated in natural seawater (SW)-marine sediments over a 3-year period.

2. Materials and methods

2.1. Seawater and marine sediments

The SW used in this study was collected from a depth of 80 m (close to the seabed) in the local fjord outside the laboratories of SINTEF Ocean, Trondheim, Norway (63°26′N, 10°24′E). The SW was transported to SINTEF's laboratories through a pipeline system made from polyethylene. The depth of this pipeline inlet (80 m) is well below the thermocline, securing a stable temperature throughout the year. The SW has a salinity of 34‰, and the water source is considered to be non-polluted and not heavily influenced by seasonal variations. The SW passes through a sand filter to remove coarse particles before entering our laboratories. Concentrations of mineral nutrients are 19 μg L^{-1} total-P, 16 μg L^{-1} *o*-PO_4-P, 130 μg L^{-1} $NO_2 + NO_3$-N, 3 μg L^{-1} NH_4-N, and <0.05 mg L^{-1} Fe (Brakstad et al., 2015).

Intertidal marine sediments consisting mainly of clay and silt were collected from a local soft-bottom beach (63°31 N, 10°15′E). SW was acclimated overnight to 20 °C while being aerated (bubbled with sterile air for 20 min), and then 400 mL of SW were mixed with 200 mL of sediment in 1 L Schott flasks. SW-sediment suspensions were allowed to settle overnight at 20 °C. $HgCl_2$ (100 mg L^{-1}) was added to the flasks used as sterile controls.

2.2. Polymer monofilaments

Blue coloured PBSAT monofilaments (diameter 0.55 mm) and blue nylon (PA 6) monofilaments (diameter 0.60 mm) were used as test and reference materials, respectively. Both PBSAT and nylon filaments were produced by S-ENPOL (Gangwon-do, South Korea). The PBSAT resin is an aliphatic-aromatic co-polyester prepared using 1.4-butanediol as an aliphatic glycol (as the base material), dicarboxylic acids such as succinic acid and adipic acid (as the aliphatic components), and dimethyl terephthalate (as an aromatic component). The PBSAT resin composition includes a colourant at 0.005–0.015 parts by weight. To improve the properties of monofilament yarn formed from the coloured resin, additives such as antioxidants and UV stabilizers may be included at 0.2–0.5 parts by weight with respect to 100 parts by weight of the PBSAT resin. The resin composition is described in patent EP3214133 A1 (Kim et al., 2017).

2.3. Experimental setup and sampling

Filaments of PBSAT and nylon were aseptically cut into 50 cm lengths, and 20 pieces of PBSAT or nylon pre-cuts were added to each flask containing SW-sediment. All flasks were wrapped in aluminium foil for light protection and incubated dark for up to 36 months at 20 °C. The test setup is described in Table SI1 and consisted of a total of 35 flasks. Flasks were sacrificed for sampling after 3, 6, 12, 24, and 36 months of incubation. Each sampling included two replicates of each gillnet filament type in natural SW-sediment, one sample of each gillnet in sterilized SW-sediment, and one SW-sediment control without gillnet. Filaments from each sampling were prepared for physical, chemical, or microbial analyses (Table SI1): Ten pieces of each filament sample replicate were wrapped in aluminium foil and stored at room temperature until used for tensile strength testing, while four pieces of each replicate were sampled for microbial, chemical, and scanning electron microscopy (SEM) analyses. Two filaments of 10 cm of each replicate sample were aseptically cut in 2 cm lengths, and five of these lengths distributed in each of two sterile microtubes (one for analyses and one for back-up). The tubes were stored at −20 °C until used for DNA

isolation The remaining 10 cm lengths of each replicate sample were dried, wrapped in aluminium foil, and stored at 4 °C for pyrolysis gas chromatography/mass spectrometry (Py-GC/MS) and SEM analyses. Unused gillnet filaments (0 months, Table S11) were also included as reference for physical and chemical analyses.

One of the test flasks from each sampling containing the remaining six pieces of PBSAT or nylon monofilaments (replicate R1; Table SI1) was placed on a shaking table and agitated for 14 days at 20 °C to simulate abrasion when abandoned gillnets on the seabed floor are exposed to sediment erosion and re-sedimentation caused by wind and currents. After the agitation period, the monofilaments were stored for tensile strength testing, as described above.

2.4. Analyses

2.4.1. Physical tests

Tensile strength tests were carried out on samples of the PBSAT and nylon monofilaments sampled during the degradation experiment using a universal tensile testing machine (H10K, Tinius Olsen TMC, Horsham, PA, USA). Samples of monofilaments (20 cm lengths, ten replicates) incubated in flasks with SW-sediment, from original PBSAT and nylon filaments not incubated in SW-sediments (ten replicates), and filaments exposed to mechanical strain (six replicates) were tested in compliance with ISO standard 1805:2006 (ISO, 2006). Tensile strength was defined as the force (kg) needed to break the sample, while elongation at break was defined as the increase in length (%) of the sample at break relative to the initial length. Surface morphologies of the filaments during the incubation period were examined with a scanning electron microscope (Zeiss EVO MA 10, Carl Zeiss AG, Oberkochen, Germany) operated at 7 keV (Karl et al., 2020).

2.4.2. Chemical analyses

Samples were analysed directly without any pre-treatment except for rinsing and drying. Samples (~25 mg) were weighed in stainless steel cups. Analysis was performed using a Frontier Multi-Shot Pyrolyzer (PY-3030D) coupled to an Agilent 7890A GC with an Agilent 5975C MS (Py-GC/MS) (Santa Clara, CA, USA). The pyrolyzer was operated in double-shot mode, with initial thermal desorption of the sample at 100–300 °C (initial time 0.10 min, 30 °C/min rate, hold 3 min) followed by pyrolysis at 600 °C (0.20 min). The interface and inlet temperatures were 320 °C, and the split ratio was 100:1. The carrier gas was helium at a constant flow of 1 mL/min. Separation was achieved using a Frontier Ultra ALLOY^{+}-5 capillary column (30 m length, 0.25 μm film thickness, and 0.25 mm internal diameter). The column oven temperature was programmed at 40 °C (2 min) and ramped up by 20 °C/min until it reached 320 °C (18 min hold). The transfer line temperature was 300 °C, the ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The ion source was operated in full scan mode (29–600 mz) at 70 eV. Additives were tentatively identified in thermal desorption chromatograms by deconvolution (Masshunter Unknowns Analysis) and comparison to the NIST17 library (NIST/EPA/NIH, 2017). A polyethylene standard was included at the start of each test series to control instrument responses and discrimination. Daily signal similarities were controlled by including measurements of unused gillnet filaments.

2.4.3. Microbial analyses

Stored replicate samples with five lengths of filaments (2 cm each) were used for DNA extraction. Genomic DNA was isolated using a ZymoBIOMICSTM DNA Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's manuals and quantified with a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and a Qubit 3.0 Fluorometer (Invitrogen, Waltham, MA, USA) with the dsDNA High Sensitivity Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Due to low concentrations of genomic DNA, the extracts were treated by a Whole Genome Amplification (WGA) Kit (REPLI-g Midi Kit), (Qiagen, 150043, Hilden, Germany) according to the producer's manuals.

Sequencing and characterization of microbial communities was carried out at the University of Bielefeld. The complete library preparation followed the standard Illumina protocol and consisted of two rounds of PCR, each followed by a clean-up step, as described by the producer (Illumina, 2013). (Amplicon, P. C. R., Clean-Up, P. C. R., & Index, P. C. R. (2013). 16s metagenomic sequencing library preparation. *Illumina: San Diego, CA, USA*). Shortly, In the first PCR round, amplicons spanning V3-V4 region of the 16S rRNA gene were generated from obtained genomic DNA using the primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′) (Klindworth et al., 2013). In the second PCR reaction, Illumina indices were attached to the generated amplicons. The indexed PCR products were quantified, normalized, and pooled. The pooled sample was sequenced on an Illumina MiSeq lane using V3 reagent kit, generating 300 bp paired end reads.

Demultiplexed sequence data received from the University of Bielefeld in FASTQ format were imported into the QIIME2 v2019.4 version of the QIIME software, as previously described (Henry et al., 2020). Diversities within samples were determined by alpha diversity measures, with Chao1 describing species numbers and the Simpson diversity index considering both species numbers and evenness. To analyse potential differences in the dynamics of microbial communities between individual samples and sample groups, multivariate statistics were used in the form of principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarity (Schroeder and Jenkins, 2018). Statistical significance of differences between sample groups was calculated by permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001). Partial least square discriminatory analyses (PLS-DA) were used to differentiate between identity of the most discriminant taxa separating the biofilms on the PBSAT and nylon filaments (Wold et al., 2001).

To illustrate the taxonomic composition of the samples (amplicon sequence variants), replicates were merged and a cut-off of 2% relative abundance was applied. Taxa below this threshold were assigned to the group “Other”.

3. Results

3.1. Physical changes

Observations by SEM were used to assess changes on the surfaces of PBSAT and nylon filaments during the incubation period in the static SW-sediment system. After 36 months, cracks and degraded areas (material loss) were observed on the PBSAT surfaces (Fig. 1A, B), whereas the nylon filaments remained mainly undamaged (Fig. 1D) when compared to the unused nets (Fig. SI2A, B). The changes on the PBSAT surfaces (i.e., axial cracks) in the natural SW-sediment system became apparent after 24 months of incubation (Fig. SI2C). PBSAT incubation in sterilized SW-sediment (Fig. 1C) also resulted in axial cracks, but not to the same degree as those observed in the natural SW-sediment system. When PBSAT filaments incubated in natural or sterilized SW-sediments were exposed to abrasion by shaking the SW-sediment suspensions for 14 days, frayed surfaces and loose fibres were observed (Fig. SI3A, B), whereas the nylon nets were largely unaffected by the treatment (Fig. SI3C, D).

Filaments were subjected to tensile testing (i.e., they were stretched until they broke) to assess strength, elongation, and stiffness. The nylon filaments showed stable results during the incubation period, with breaking strengths between 9 and 10 kg for all treatments (standard deviations ± 0.34 kg) and elongation of 22–29% ± 1.93%. Neither incubation in SW-sediments nor the abrasive forces imposed by shaking the SW-sediment suspension significantly reduced breaking strength or elongation of the nylon filaments (Fig. 2A, B). However, breaking strength and elongation of the PBSAT filaments were highly affected by incubation time (Fig. 2C, D). Average breaking strength was reduced from 10 kg to 2.3 kg after incubation in natural SW-sediments for 36

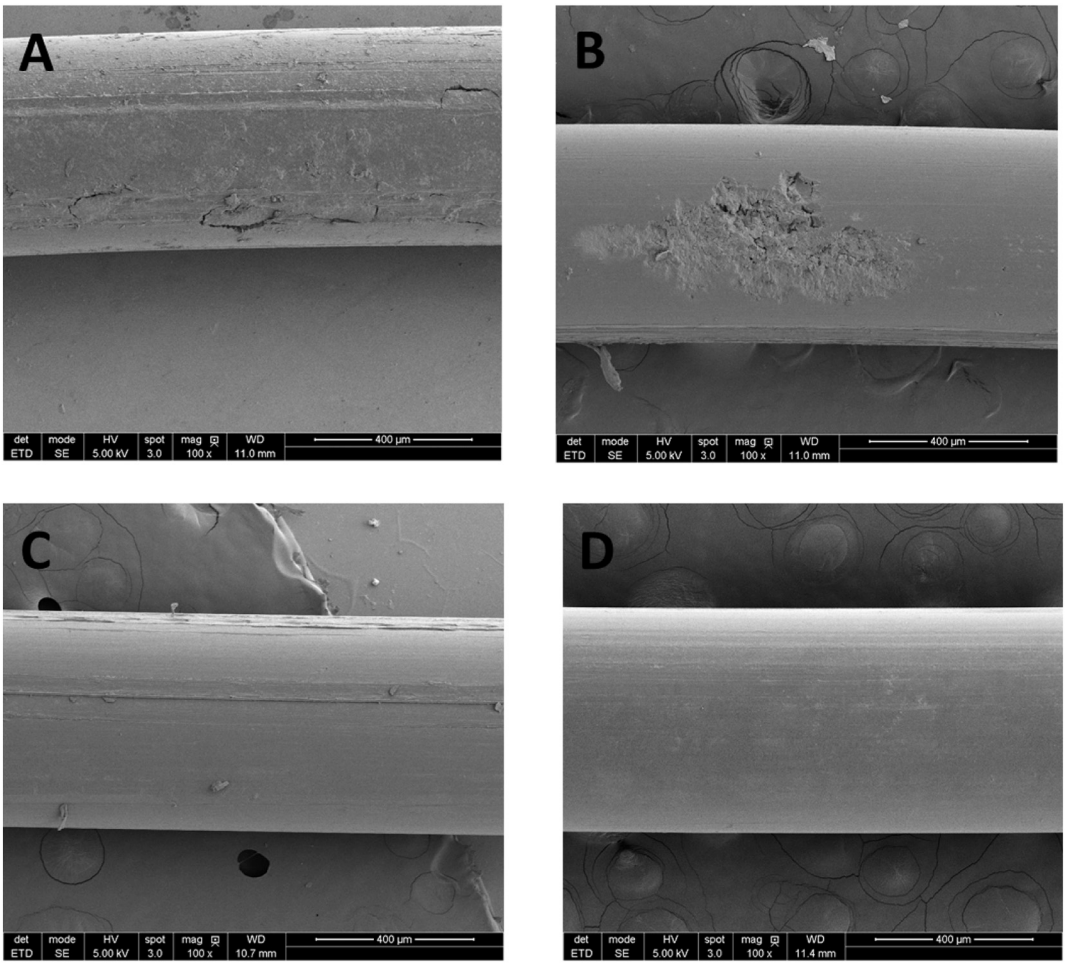

Fig. 1. SEM analyses of PBSAT (A–C) and nylon (D) gillnets after 36 months of incubation in SW-sediment systems. The nets were incubated in static systems with normal (A, B and D) or sterilized SW (C).

months and to 4.3 kg in the sterilized system (standard deviations ranging from 0.8 to 2.2 kg). Elongation of the PBSAT filaments was reduced from 25% to 5.9% ± 0.8% and to 13.0% ± 2.1% in the natural and sterilized SW after 36 months, respectively. The breaking strengths and elongations were not affected by the mechanical strains caused by shaking in the natural and sterilized SW-sediment systems (Fig. 2).

Both SEM and tensile strength tests showed negligible to limited effects of the 3-year incubation on the properties of nylon filaments, whereas the PBSAT properties were highly affected. Additionally, both SEM and tensile strength tests revealed differences between incubation in natural SW-sediment compared to sterilized SW-sediment, suggesting a potential impact of SW and/or sediment microbial communities on the strength and elongation properties of PBSAT filaments.

3.2. Microbial characterization of filament biofilms

Filament surfaces were swabbed to analyse their microbial biofilms. These samples generally contained DNA concentrations that were too low for reliable 16S rDNA microbiome analyses, so WGA was performed on all samples. DNA concentrations were generally higher on the PBSAT filament surfaces than on the nylon surfaces during the first 24 months of the experiment, but the results did not differ significantly at 36 months ($P > 0.05$; unpaired *t*-test). Negligible DNA concentrations in the sterilized SW-sediments were maintained for the first 12 months, while DNA concentrations in WGA samples collected after 24 and 36 months of incubation were comparable to those of the natural SW-sediment samples (Fig. SI4). Therefore, sterilization with $HgCl_2$ only suppressed microbial growth and did not sterilize these samples completely.

Statistical analyses of 16S rDNA microbiome data (alpha diversities) showed significantly higher species numbers ($P < 0.05$) in natural versus sterilized SW-sediments (Fig. SI5), demonstrating the survival of a limited number of species in the systems with added $HgCl_2$. Alpha diversities did not differ significantly ($P > 0.05$) between biofilm communities on PBSAT and nylon filaments, although the diversities were slightly higher for the PBSAT filaments, as shown by Chao1 and Simpson indices (Fig. SI6). Alpha diversity analyses of microbial succession in natural SW-sediment samples over time showed only small, non-significant ($P > 0.05$) differences in the taxa present (data not shown).

The differences in biofilm communities on PBSAT and nylon filaments in natural SW-sediments were compared using beta-diversity analysis, which assesses diversity between samples. A two-dimensional PCoA plot of the beta-diversities showed that the differences in microbial community compositions between the two types of gillnet filaments were significant ($P < 0.05$, PERMOVA) and were not influenced by uneven sample dispersion (Fig. 3). PLS-DA showed well-separated clusters of samples between PBSAT and nylon biofilms (Fig. SI7), suggesting that each polymer has unique taxonomical features.

Fig. 4 shows the microbial community members of the gillnet filament biofilms that were present with relative abundances >10%. The SW at the start of the experiment was dominated by Gammaproteobacteria affiliated with the families *Vibrionaceae* and *Saccharospirillaceae*, with 34% and 49% relative abundances, respectively (Fig. 4), but neither of these families was associated with the abundant members of the gillnet biofilms. Only two of the abundant taxa occurred in the

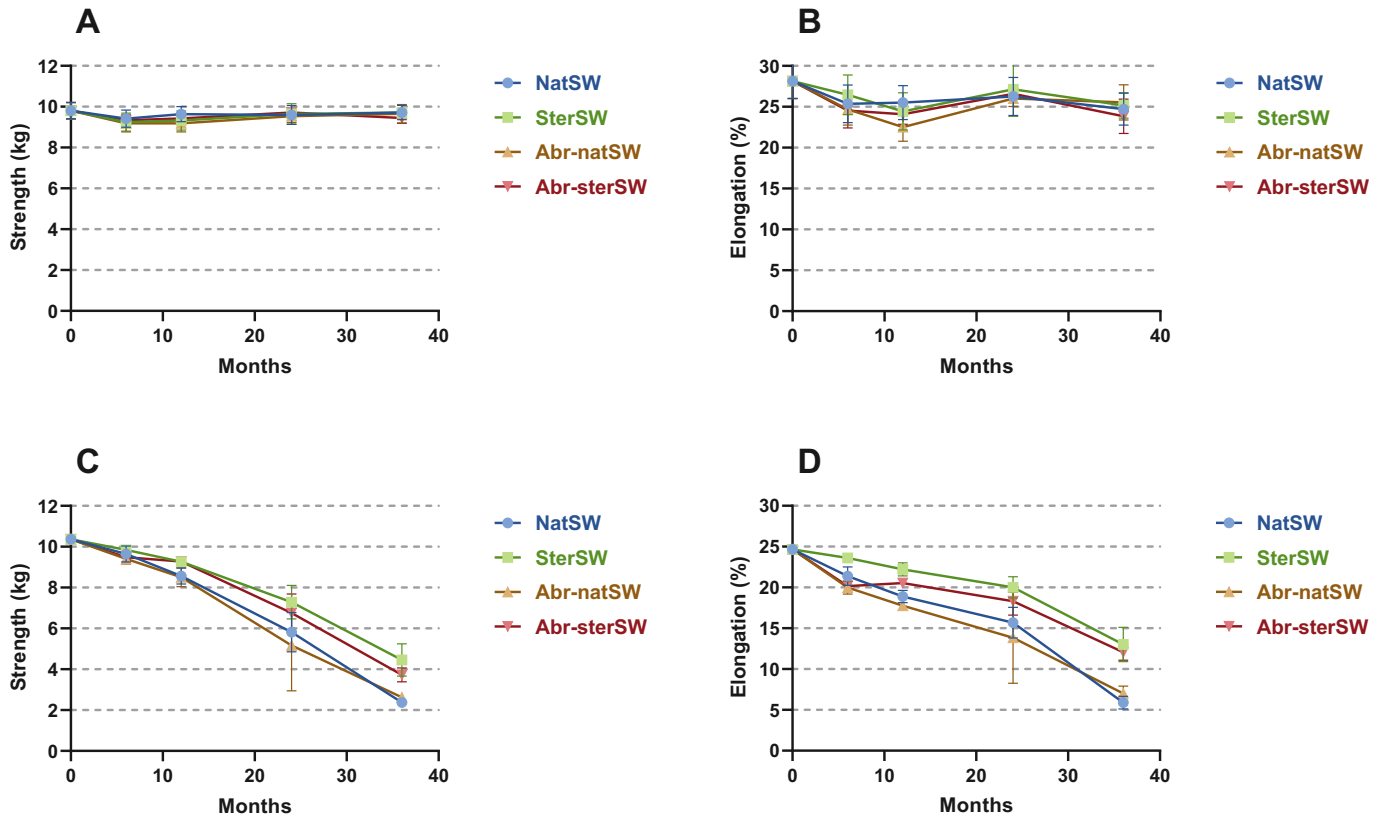

Fig. 2. Tensile testing of nylon (A and B) and PBSAT (C and D) filaments during the 36 month's incubation period, using 10 replicates for measurements. Results are shown as the filament strength in kg (A and C) and % elongation at break (B and D). The results are shown for filaments incubated in natural SW-sediment (NatSW), sterilized SW-sediments (SterSW), and filaments agitated for 14 days in natural (Abr-natSW) or sterilized (Abr-SterSW) suspensions of SW and sediments. The error bars represent standard deviations of replicate measurements.

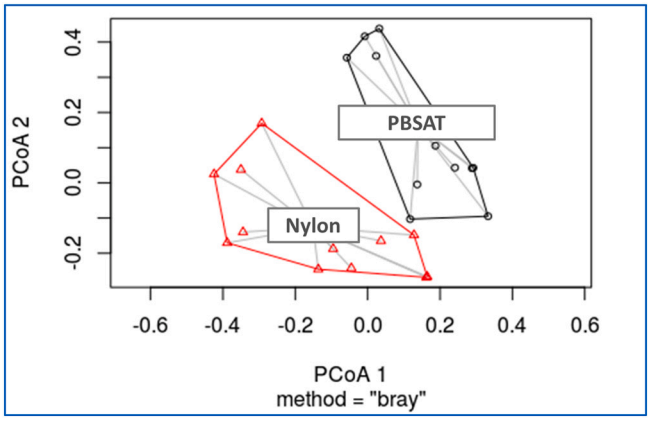

Fig. 3. Beta-diversity comparison between microbial communities on biofilms from PBSAT and nylon gillnet filaments in natural SW-sediment samples based on Bray-Curtis dissimilarities.

samples from both types of gillnets: unassigned bacteria Babeliales (6%–20% in nylon films between 18 and 36 months and 13%–22% in PBSAT biofilms after 36 months) and amplicon sequence variants assigned to the uncultured marine Alphaproteobacterium NRL2 (4%–29% in nylon biofilms and 6%–23% in PBSAT biofilms, both after 6–12 months of incubation).

The microbial succession in the nylon filament biofilms was associated with bacterial groups remaining abundant through parts of the 36-month incubation period, including the Chlamydiales *Simkaniaceae* (2%–55% relative abundances during 6–36 months of incubation), the Alphaproteobacteria *Sphingomonadaceae* (5%–31% relative abundances between 6 and 24 months), and an unassigned Actinobacteria bacterium IMCC26256 (4%–45% relative abundances between 18 and 36 months of incubation). Other taxa were present during shorter periods, particularly as late members of succession (e.g., uncultured Alphaproteobacterium *Candidatus* Puniceispirillales (19% relative abundance after 24 months), *Simkaniacea* (55%) and Dehalococcoidia S085 uncultured bacterium (24%) after 36 months; and the Gammaproteobacteria *Diplorickettsiaceae* (relative abundance 38%) after 36 months).

Some taxa were dominant members of the PBSAT biofilms over extended periods of time, including taxa affiliated with two Gammaproteobacteria (*Cellvibrionaceae* with 2%–15% relative abundances from 6 to 36 months and an unassigned clade KI89A with 7%–57% relative abundances after 6–36 months) and two Alphaproteobacteria (*Hyphomicrobiaceae*) with 8%–16% relative abundances from 6 to 36 months and *Magnetospiraceae* with 7–14% relative abundances from 12 to 24 months of incubation. The PBSAT biofilms also contained amplicon sequence variants that were abundant for shorter periods of time, such as those affiliated with Gammaproteobacteria of the *Vibrionaceae* family (37% relative abundance in one replicate after 18 months), uncultured Gammaproteobacteria EC3 (13% relative abundance), and the unassigned Babelliales (13%–22% relative abundance after 36 months).

3.3. Chemical characterization of filaments

Changes in monofilament polymer structures were analysed by double-shot mode Py-GC/MS. Thermal desorption GC/MS-analyses of the filaments performed at 300 °C identified mainly surface-related compounds on filaments. A selection of these compounds was tentatively grouped into 1) monomers, 2) potential degradation products, and

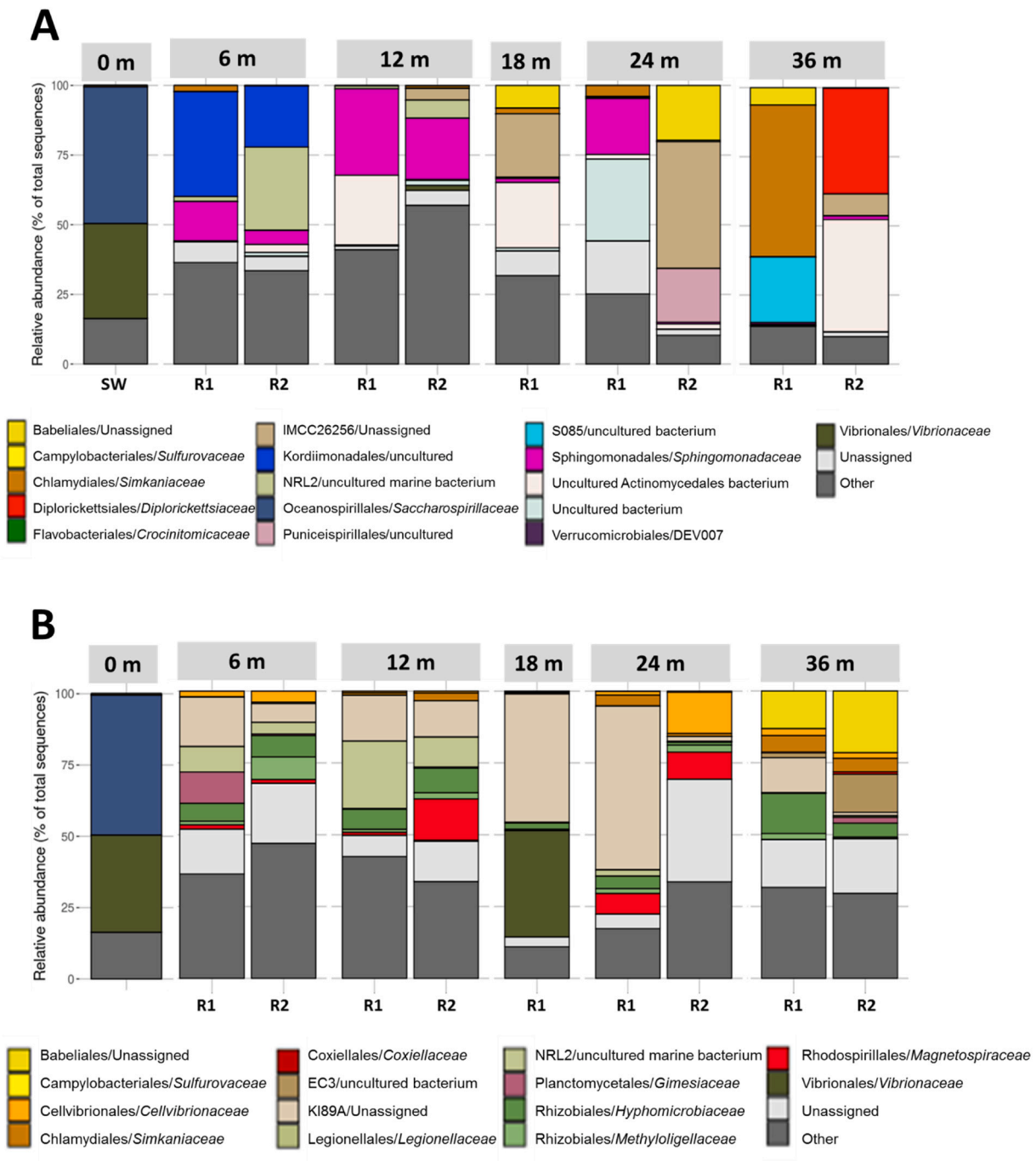

Fig. 4. Microbial community composition of WGA amplicons from biofilms on nylon (A) and PBSAT (B) filaments (cut-off of 10% in relative abundance). Column headings are indicating incubation month. Data of two replicates (R1 and R2) are shown, expect for samples of SW at the start of the experiment (0 m) and after 18 months of incubation.

3) additive chemicals. Fig. SI8 shows the relative distribution of potentially identifiable compounds in nylon and PBSAT filaments. The most significant peak for nylon filaments belonged to caprolactam (CAS 105–60-2, > 97% match to NIST17), which is the monomer used for the synthesis of polycaproamide (nylon-6), and another abundant peak was assigned to 1,8-diazacyclotetradecane-2,9-dione (CAS 5776-79-4, > 85% match to NIST17) (Fig. SI8A), which is a nylon-6 cyclic oligomer and likely a synthesis by-product. Two additives (dioctyl terephthalate CAS 6422-86-2 and 2-heptadecyl-2-imidazoline CAS 105-28-2) were also tentatively identified in the nylon filaments. Comparison of relative responses between natural and sterilized SW-sediment microcosms during the incubation period showed significantly different ($P < 0.05$: paired *t*-tests) and higher relative responses of caprolactam, diazacyclotetradecanedione, and dioctyl terephthalate in the sterilized samples compared to the natural samples during the first 24 months (Fig. 5). However, response reductions were detected in the sterilized controls at 36 months, likely due to microbial growth in the sterilized systems at the end of the experiment (Fig. SI4). No decreases of heptadecyl imidazoline responses were measured in the experiments (Fig. 5).

Thermal desorption chromatography identified nine compounds in the PBSAT filaments (Fig. SI8B). The quantitatively dominant peak was identified as 1,6,11,16-tetraoxacycloicosane-2,5,12,15-tetrone (CAS 110365-01-0), a cyclic oligomer of PBSAT. This compound and some of the others tentatively identified in the filaments (spectra matching

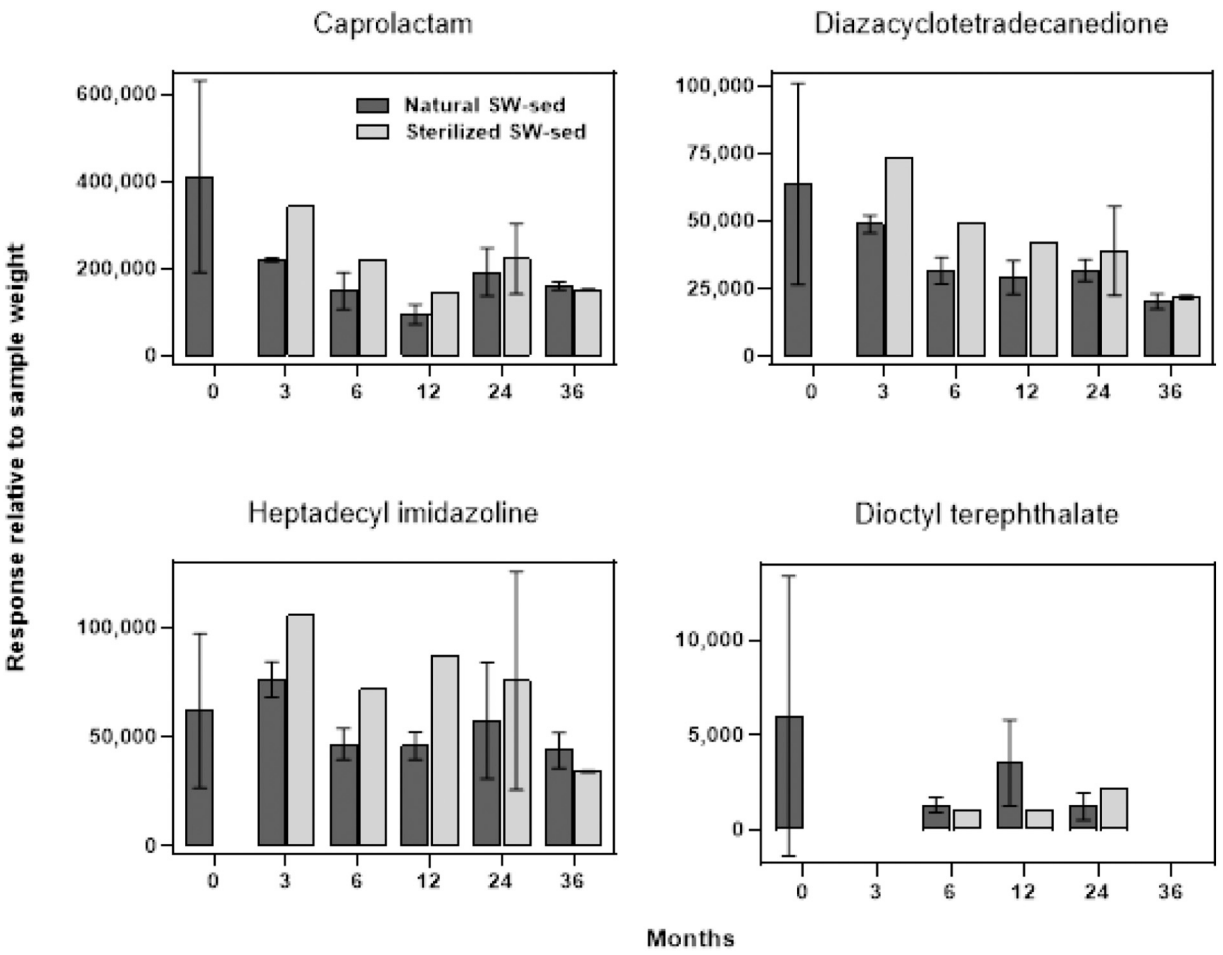

Fig. 5. Relative responses of compounds potentially identified by thermal desorption GC/MS analyses of nylon filaments in biotic and sterilized SW-sediment (0–36 months). The error bars represent standard deviations from measurements of 3 replicates.

isomeric identities of succinimide, succinic anhydride, dibutenylester succinic acid, butyl adipate, and heptyl dioxepane) could be explained as either synthesis by-products or degradation products. Some additive compounds, including two phthalates (diethyl phthalate and dioctyl isophthalate) and the colourant violet 13, were also identified in the PBSAT filaments (Fig. SI8). Several compounds showed temporary increases followed by decreases in relative concentrations over time (Fig. 6). Significantly increased responses from experimental start (day 0) to 3 months of incubation ($P < 0.05$, paired *t*-test) were detected in the SW-sediment microcosms for tetraoxacycloicosane tetrone, succinic anhydride, heptyl dioxepane, dibytenylester succinic acid, succinimide, and butyl adipate (Fig. 6). The relative responses in the sterilized controls were mainly lower than those in the natural samples after 3 months, but the differences were not statistically significant ($P > 0.05$, paired t-test). However, the higher responses of these compounds in the biotic samples could indicate that they were formed as biodegradation products during the 3 month period. Concentrations of some of the compounds, including tetraoxacycloicosane tetrone, heptyl dioxepane, and butyl adipate, decreased significantly ($P < 0.05$, one-way analysis of variance (ANOVA)) from 3 to 36 months of incubation (Fig. 6). Succinimide concentration continued to increase until 12 months of incubation, followed by a decrease between 24 and 36 months, although the decrease was not statistically significant ($P > 0.05$, one-way ANOVA). Other compounds also showed decreased responses in the SW-sediment experiment over time, such as diethyl phthalate, dioctyl isophthalate, and violet 13. These decreases could be due to leaching or biodegradation. Decreased responses of several compounds were also detected in sterilized controls over time, particularly late in the biodegradation period (Fig. 6), which was in line with contamination of these samples indicated by increased DNA concentrations (Fig. SI4). Some compounds did not show relative response changes during the experimental period in the SW-sediment experiment, including dibutenylester succinic acid and succinic anhydride (Fig. 6), which indicates negligible biodegradation.

Pyrolysis analyses of the filaments were performed at a temperature of 600 °C to delve deeper into the filaments. However, the pyrolysis step did not generate changes in the chromatograms over the 36 month experimental period (Figs. SI9 to SI10).

4. Discussion

In this study, a microcosm experiment was performed to incubate gillnet filaments over a period of 36 months in a system containing natural SW and marine sediment to represent a scenario in which lost gillnets end up on the seabed. Conventional nylon nets are expected to persist in the marine environment, which poses a high risk of ghost fishing by abandoned nets. In contrast, PBSAT nets are considered to be degradable in the marine environment (Tokiwa et al., 2009). However, biodegradation of PBS and PBAT has mainly been confirmed by respirometric tests or by weight loss or reductions in tensile strength (Kim et al., 2005; Kijchavengkul et al., 2010; Wang et al., 2015; Nakayama et al., 2019; Yamano et al., 2019), and no direct proof of microbial bond breaking or de-polymerization in natural marine systems has been reported to date.

The nylon gillnet filaments tested in this study showed stabile breaking strength and elongation during the 36 months of incubation in

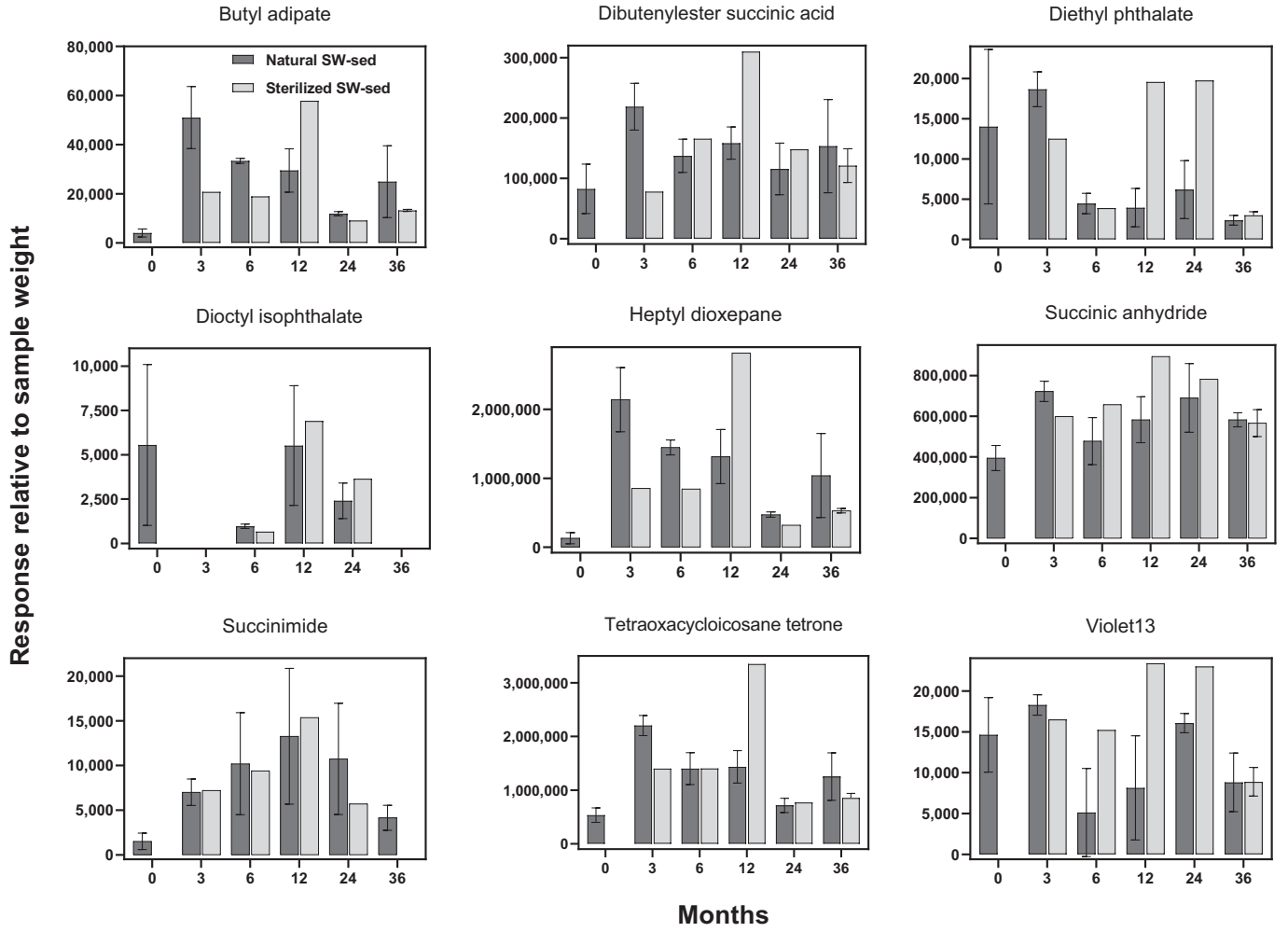

Fig. 6. Relative responses of compounds potentially identified by thermal desorption GC/MS analyses of PBSAT filaments in biotic and sterilized SW-sediment (0–36 months). The error bars represent standard deviations from measurements of 3 replicates.

SW-sediment as well as intact surface morphologies when observed by SEM. The chemical analyses by Py-GC/MS and thermal desorption GC/MS did not show any attacks on the polymeric structures of the nylon filaments, and only potential nylon-6 synthesis monomers and by-products (caprolactam and 1,8-diazacyclotetradecane-2,9-dione) and additives (dioctyl terephthalate and 2-heptadecyl-2-imidazoline) were detected. Of these compounds, the products associated with nylon-6 seemed to be slowly biodegradable in the SW-sediment microcosm. The lack of polymer degradation and intact surface morphologies of the nylon filaments likely explain the tensile strength stability of these filaments. For widely used high-molecular plastics like nylon, polystyrene polyvinylchloride, and polypropylene, few or no enzymes have been identified to date which may be associated with polymer degradation (Emadian et al., 2017), although some fungi have been reported to disintegrate nylon-6 (Friedrich et al., 2007). Marine bacteria have been associated with degradation of nylon-6 at high seawater temperature (35 °C), resulting in molecular weight reductions (Sudhakar et al., 2007), Yamano et al., who conducted oxygen consumption tests to study polyamide (nylon) biodegradation in artificial SW at 27 °C, reported 30% biodegradation after 4 weeks, and they isolated nylon-degrading bacteria (Yamano et al., 2019).

While the nylon filaments were mainly unaffected by the 3 year incubation in SW-sediments, the PBSAT filaments exhibited decreases in strength and elongation, with lower strength and elongation in natural versus sterilized SW-sediments. These changes in filament properties were associated with changed surface characteristics, which were visible as cracks and degraded areas on the PBSAT surfaces in SEM images. Filaments were also affected in sterilized SW-sediments, but to a lesser extent than in natural SW-sediments. The reduced tensile strength in the sterilized system could partly be the result of contamination, as sterility was not maintained between 12 and 36 months of incubation. Field test comparisons of nylon and PBSAT filaments have also shown reductions in PBSAT versus nylon tensile strength. For instance, PBSAT gillnets exhibited 26% lower tensile strength than nylon gillnets after 25 months of immersion in Norwegian SW (Su et al., 2019). The use of these first-generation degradable gillnets may therefore be a compromise between reduced fishing efficiency and improved environmental behaviour in the form of reduced ghost fishing of abandoned nets.

Biodegradation of gillnets is often evaluated through indirect methods, such as weight loss, tensile strength and stiffness reduction, and visual changes in surface structure visualized by SEM analysis. Biodegradation of plastic polymers in seawater is mainly associated with disintegrated fragments, which may sink in SW due to their density (Tosin et al., 2012). Gillnet filaments were therefore incubated in an SW-sediment microcosm system in this study. While ultimate biodegradation methods, like biochemical oxygen demand or CO_2 evolution (ISO, 2019), do not discriminate between biodegradation of polymers and small additives, primary biodegradation methods with target-specific analyses are more informative for assessing polymer biodegradation. Double-shot mode Py-GC/MS was used to analyse gillnet polymer structures. (Hendrickson et al., 2018) previously used this method to analyse plastics abundances and compositions in environmental

samples. In the double-shot mode, only the initial thermal desorption, and not the pyrolysis step, resulted in differences in target analyte abundances during our 3 year experimental period. These results indicated degradation of the polymer structure in the surface layers and not in the deeper layers of the gillnet monofilaments.

In nylon nets, depletion of caprolactam and diazacyclotetradecanedione could be related to slow biodegradation of these compounds, and they are probably present in the gillnets as residues from the nylon-6 synthesis process (Kulkarni and Kanekar, 1998). In the PBSAT filaments, some compounds showed higher relative abundances over time in natural versus sterilized SW. These included tetraoxacycloicosane tetrone, succinic anhydride, heptyl dioxepane, dibytenylester succinic acid, succinimide, and butyl adipate. Aliphatic-aromatic co-polyesters like PBSAT (Fig. SI1) are reported to be biodegraded with the aliphatic polyester expected to degrade faster than the aromatic entity (Lucas et al., 2008). Typically, succinic acid monomers are detected as the first products of depolymerase-induced biodegradation of aliphatic-aromatic co-polyesters. The subsequent products are 1,4-butanediol and adipic acid (Shah et al., 2013). Shah et al. (2014) reported that biodegradation of aliphatic-aromatic co-polyesters is slower than of aliphatic polyester. Therefore, the succinic acid, dibutyl succinate, and dibutyl adipate detected in this study presumably were biodegradation products of the aliphatic part of the polymer. Succinic anhydride, which is used as coupling agents for linking biofibers through hydrogen bonding or esterification, is likely a residue from polymer synthesis (Moussa and Young, 2012; Siyamak et al., 2012). Tetraoxacyclotetracosane tetrone, a compound related to tetraoxacycloicosane, has been detected in compostable adhesives and is thought to be generated by a reaction between 1,4-butanediol and adipic acid (Ramos et al., 2019). Additional compounds detected by the Py-GC/MS analyses included diethylphthalate, dioctyl isophthalate, and violet 13. While violet 13 is a common colourant used in plastics and other products (Arfania et al., 2017), the presence of phthalate compounds may indicate degradation of the aromatic entities of the PBSAT polymer (Shah et al., 2014). Diethylphthalate in particular showed initial accumulation, followed by a decline in concentration in normal SW when compared to the sterilized controls, indicating initial release from the polymer followed by further biodegradation of the compound.

The differences in the degradation patterns between the nylon and PBSAT gillnets were also reflected by the differences in relative abundances of microbial communities in the gillnet biofilms. The microbial communities in the biofilms differed significantly between the gillnets, as shown by beta diversity analyses. Some of the abundant groups in the nylon biofilms have been reported to occur in marine biofilms on plastics and microplastics, e.g. members of the families *Simkaniaceae* and *Sphingomonadaceae* (Rogers et al., 2020). Members of the *Sphingomonadaceae* are also associated with hydrocarbon and pesticide biodegradation and have been reported to be abundant in biofilms on polyethylene (Oberbeckmann and Labrenz, 2020). However, their role in potential polymer biodegradation is unclear. Dehalococcoidia have been reported in microplastic biofilms, particularly when enriched with contaminants like polychlorinated biphenyl (Rosato et al., 2020). Abundant members of the PBSAT biofilms have also been reported to occur in plastic biofilms. For instance, Pinto et al. reported that members of the cellulolytic bacterial family Cellovibrionaceae were dominant in marine polyvinyl chloride biofilms under dim conditions (Pinto et al., 2019). Members of the *Vibrionaceae* have been detected as abundant members of plastic-associated biofilms, in particular on floating biofilms in the North and Baltic Seas, whereas they were less abundant on the plastic debris from the North Pacific gyre (Oberbeckmann et al., 2018; Rogers et al., 2020). Although we propose that members of the PBSAT biofilms were involved in polymer biodegradation, no reports to date have described any biodegradation mechanisms of aliphatic-aromatic co-polymers associated with any of the abundant microbial groups. However, few studies of microbial degradation mechanisms of these polymers in the marine environment have been conducted (Shruti and Kutralam-Muniasamy, 2019).

5. Conclusions

In this study, several key factors important for the degradation of monofilaments used in conventional nylon and biodegradable PBSAT gillnets were compared during a 3 year study conducted in a natural SW-sediment system. The nylon monofilaments remained essentially unaffected during the study period, whereas the PBSAT filaments showed signs of degradation, including surface cracks, abrasion damage, and decreased tensile strength and elongation. The PBSAT monofilaments also were affected by physical strain caused by sediment erosion, which is relevant for abandoned fishing gear on the seabed and in the littoral zone. The deterioration identified by SEM analyses and tensile strength tests may also be associated with surface biodegradation caused by biofilms accumulating on the gillnet filaments. This premise was supported by the finding that SW sterilization resulted in less physical deterioration of the PBSAT gillnets. Although the abundant bacterial groups in the PBSAT biofilms were not associated with bacteria reported to degrade PBSAT polymers, we expect members of the abundant groups to participate in biodegradation.

Even though PBSAT gillnets will have shorter lifetime than conventional nylon nets and may result in reduced catch efficiency over time, the environmental benefits of using these nets are important. The physical and chemical deterioration expected to occur with PBSAT nets may reduce their lifetime and the time of ghost fishing by several years, which could be of great environmental significance considering the high numbers of abandoned nets worldwide.

CRediT authorship contribution statement

Credit role	OG Brakstad	L Sørensen	S. Hakvåg	HM Føre	B Su	M Aas	D Ribicic	E Grimaldo
Conceptualization	X	X						X
Data curation	X	X		X				
Formal analyses		X		X	X	X		
Funding acquisition								X
Investigation	X			X		X	X	
Methodology	X	X		X	X	X	X	
Project Administration								X
Resources	X			X				X
Software		X	X		X		X	
Supervision	X	X		X				
Validation	X	X	X		X	X	X	
Visualization			X					
Writing – original draft	X	X		X	X	X	X	X
Writing – review and editing	X		X		X			

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study received financial support from the Research Council of Norway (grant number: 300008). Kjersti Almås at SINTEF Ocean was responsible for the Py-GC/MS analyses, and Huiting Jin at SINTEF Industry, Dept. Materials and Nanotechnology conducted the SEM analyses. We would also like to thank Julia Hassa and Dr. Tobias Busche from the CeBiTec team at Bielefeld University for their contributions to library preparation and sequencing of amplicons.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2022.113759.

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