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# MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

**„The influence of solar radiation on the microbial colonization of different Plastic types in the northern Adriatic Sea"**


verfasst von / submitted by

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## Abstract

Plastic pollution is one of the biggest threats to the marine environment with an annual input of plastic waste estimated to be between 4.8 and 12.7 million metric tons. After entering the oceans, plastic debris is rapidly colonized by microbes, resulting in the formation of complex biofilm structures by microbial communities and other biofouling species on the plastic´s surface. The influence of solar radiation on colonization patterns of different plastic types is largely unknown. In this study, pristine plastics of five different types (low density polyethylene, PE; high density polyethylene, HDPE; polypropylene, PP; and polyvinylchloride with two different additives, PVC DEHP, PVC DINP) as well as glass slides (as controls) were incubated in the surface waters of the northern Adriatic Sea off the coast of Rovinj, Croatia, for up to two months to investigate the influence of solar radiation on the establishment of microbial biofilms. The plastics were incubated under full solar radiation and dark conditions and sampled after one week, one month and two months of incubation. The biofilm structure over time was analyzed using scanning electron microscopy. Also, the phylogenetic composition of the biofilm community was investigated. The incubated plastic types were covered with a diverse biofilm, showing variations in thickness and coverage between samples, conditions and exposure times. The HDPE and glass samples showed nearly no signs of biofilm after one week of incubation, but were covered with a diverse and dense biofilm at the later stages of incubation. The PVC DEHP samples exhibited a thick and diverse biofilm right from the initial time of sampling onwards. Phylogenetic analysis of the biofilm revealed differences in community composition between solar radiation exposed and dark treatments and among different plastic types. The bacterial community composition of the plastic associated biofilm was different from that in the ambient water. No compositional differences, however, were detected between the incubated plastics and the glass slides. The mean Shannon Index at the beginning and end of the experiment and the species richness at the end of the experiment were significantly higher in plastics kept in the dark than in those exposed to solar radiation. The community composition of the PVCs clearly differed from that of the other plastic types. Furthermore PVC DEHP showed a significantly lower mean species richness after one week of incubation compared to the other substrates, indicating a possible influence of surface roughness and leaching plasticizers on biofilm establishment.

Various known hydrocarbon/oil-degrading taxa were only found in the biofilm but not in the ambient seawater, indicating the potential of plastics serving as a carbon source for some bacterial taxa.

## Introduction

Plastic pollution of marine environments represents a major threat for marine life. Synthetic polymers like plastics were first produced around 100 years ago and production increased exponentially ever since[1]. Plastics are high molecular weight synthetic polymers, consisting of long hydrocarbon chains that can possess different side groups[2,3]. Plastic is produced using organic materials like cellulose, natural gas and crude oil[4]. During the production process other substances like stabilizers and plasticizers e.g. bisphenol A, phthalates or nonylphenols are added to make the plastics more persistent to UV radiation, heat or just to ensure the plasticity of the material. Phthalates are additives mostly used in the production of polyvinylchloride (PVC), which can make up between 10%-60% of its total weight[5,6]. Phthalates, like Di(2-ethylhexyl) phthalate (DEHP) and Diisononyl phthalate (DINP), which are the most commonly used additives, can be leaching from the plastics into the surrounding environment since they are not incorporated in the polymer structure itself [6,7]. These additives can have toxic effects on organisms by acting as endocrine disrupters or carcinogens but the full impact still needs further investigations [8].

Studies have reported ingestion and entanglement of marine organisms caused by plastic waste in the oceans[9]. The persistence and potential threats of plastic debris to wildlife and the humans are a rising concern, especially given the increase in plastic manufacturing, which just in 2015 alone resulted in a global production of 322 million metric tons [4]. The amount of plastic entering the ocean is estimated to be between 4.8 to 12.7 million metric tons per year[10]. The factors determining the input of plastic waste to the oceans is human population size and waste management systems in the respective countries. The main fraction of plastics reaches the ocean via streams and rivers [10].

Over time, large plastic pieces break down to smaller pieces, called microplastics, which are defined as pieces smaller than 5mm in length [11]. Microplastics can be split into two groups, primary and secondary microplastics, depending on their origin. Primary microplastics are very small plastic beads, often used in cosmetic products, abrasion from car tires or pre-production resin pellets [12]. Secondary microplastics are those resulting from

physical or biological degradation of larger pieces [13,14]. Ingestion of these microparticles by animals such as fishes or seabirds often leads to death due to plastic accumulation in the intestinal tract[15,16]. In the year 2014, it was estimated that 15 - 51 trillion pieces of microplastics were afloat on the ocean surfaces, which equals 93,000 – 236,000 metric tons [11,17]. Nevertheless, the fate of plastic debris in the oceans is still unknown since the number of floating microplastics accounts only for around 1% of the amount entering the ocean each year [18,19]. Plastic debris has been found all around the globe.

Due to its positive buoyancy and the oceans current patterns, plastic accumulates in the main convergence zones - the ocean gyres. There are in total five gyres in the world's oceans - two in the Atlantic, two in the Pacific and one in the Indian Ocean. Several studies showed the dispersion of plastic debris in the Atlantic and Pacific gyres[20,21]. It was estimated that around 40% of all microplastics accumulate in the North Pacific gyre[11]. The Mediterranean Sea is also highly impacted by plastic debris, as indicated by the highest microplastic abundance among the world's oceans[22]. The Mediterranean Sea is one of the most frequented water bodies and the largest enclosed sea worldwide[22]. The problem of plastic pollution was thought to affect primarily the northern hemisphere since the human population density is greater there compared to the southern hemisphere. Recent studies, however, have documented the existence of microplastics in more remote areas like the Southern Ocean as well as in Arctic polar waters[23,24]. Plastic particles act as a float for microbes and other biofouling biota, while persisting longer than most natural substrates in the marine environments [25]. Furthermore, studies indicated the potential of plastics to act as a transport vehicle for neobiota and persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) or dichlorodiphenyltrichloroethane (DDT) [26].

Providing a new floating substrate with a hydrophobic surface, it was shown that microbial communities, which establish diverse biofilms of autotrophs, heterotrophs and predatory organisms, rapidly colonize plastic debris. This unique environment was coined "plastisphere"[25,27]. Studies demonstrated that microbial biofilm communities differ in composition with regards to biogeography, season and polymer type[28,29]. Biofilm establishment proceeds rather fast and is visible on pristine plastics after one week of incubation in the ocean[30]. It has been suggested that besides the biofouling community, potential harmful algal or pathogenic microbial genera like Vibrio could use the plastic pieces as a vector[31,32]. Biofilm establishment on plastic debris results in a change in buoyancy and

sinking of the particles. This could be a possible explanation for the disparity between the estimates of plastic particles entering the ocean and the number of floating particles[33]. Recent studies indicated the biodegradation of plastic by marine bacteria and fungi, suggesting possible ways to reduce the plastic debris in aquatic environments[34,35]. Due to their complex chemical structure, plastics are very persistent and generally need to undergo more than one type of degradation before being taken up by microorganisms, such as thermal, mechanical, photolytic and chemical degradation[36].

Biodegradation pathways were shown for polyethylene terephthalate (PET) by a bacterial strain that produces two enzymes capable of hydrolyzing PET using the arising products as carbon source [34]. Assimilation by a marine fungus was so far only demonstrated by morphological and molecular changes of the plastic[35]. Recently, a new group of hydrocarbon-degrading bacteria was described - the marine obligate hydrocarbonoclastic bacteria, or short "OHCB"[37,38]. After oil spill events, this bacterial group was observed forming excessive blooms since they are key players in the degradation of crude oil and other oil constituents[37,38]. Crude oil is the most important constituent of plastic production and is, as well as plastic, based on a hydrocarbon structure[4,39]. Therefore, both materials share bacterial taxa involved in their biodegradation[25,39,40]. Bacteria dominating the bloom communities belong to the following genera: *Alcanivorax*, *Marinobacter*, *Thalassolituus*, *Cycloclasticus*, *Oleiphilus*, *Alteromonas* and *Pseudoalteromonas*[37,38,41,42]. The specific community composition can vary and depends on abiotic factors like latitude/longitude, salinity and temperature [37]. These hydrocarbon-degrading taxa are affiliated with Alpha- and Gamma-Proteobacteria, most of them with the latter[37]. Due to the positive buoyancy of the most common plastic types (LDPE, HDPE, PP), most of the plastic debris is floating at the oceans surface and therefore constantly exposed to solar radiation[43]. However, the possible influences of solar radiation on physical degradation or biofilm establishment are largely unknown.

Several studies investigated the influence of solar radiation on microbial communities and their activity [44–47]. It has been observed that microbial communities show different sensitivities to solar radiation [44,45,48]. After the Deep Water Horizon oil spill in the year 2010, a study found that natural sunlight has an influence on the oil-degrading bacterial community [49]. They observed that specific genera like *Alcanivorax, Formosa, Pseudomonas*, etc. were particularly abundant under dark conditions whereas other genera like

*Alteromonas, Marinobacter, Bartonella,* etc. were dominant only in the incubations with solar radiation present [49].

Compared to phytoplankton, small organisms like bacterioplankton are especially sensitive to solar radiation since their low volume:surface ratio makes it inefficient to use self-shading mechanisms. The mass and energy they have to invest in pigment production is increasing with decreasing cell size[50]. A study testing the resistance of bacterioplankton to solar radiation discovered that genera like *Pseudoalteromonas* and *Alteromonas* are highly resistant to solar radiation albeit not being pigmented [51]. Furthermore, this study found no relation between pigmentation and the influence of ultraviolet (UV) irradiation. It was also shown that there is no relationship between the resistance to solar radiation and the habitat from where the microorganisms were sampled (surface microlayer or deeper water masses). Overall, solar radiation exhibits an inhibitory effect on bacterial activity as determined by leucine or thymidine incorporation[46,47]. UV-B radiation (280nm-320nm wavelength) has the most harmful effect on bacterial activity and exoenzymatic activity compared to UV-A (320nm-400nm) or photosynthetically active radiation (PAR - 400nm-750nm). UV-B radiation can damage DNA by leading to the formation of thymine dimers and to the degradation of extracellular enzymes[52]. Due to its short wavelength, UV-B only penetrates the first 25m of the water column in the oligotrophic open ocean. UV-A and PAR penetrate the water column deeper and allow bacteria to recover from UV-stress by activating repair mechanisms[53]. There are three different DNA repair mechanisms: photoenzymatic repair (PER), nucleotide excision repair and post replication repair[54]. The latter two require ATP while PER is activated by UV-A and PAR radiation. The harmful effect of UV-radiation on some species leads to a change in community composition in surface waters, but this change is also influenced by seasonal variability of solar irradiance[20,55]. It was observed that solar irradiance directly influences biofilm communities. UV-radiation and PAR directly control the biofouling community by inhibiting sensitive species from developing and supporting more resilient species [56].

This study focuses on the influence of solar radiation on microbial biofilm establishment on pristine plastics. The goal of this study was 1) to examine how solar radiation influences biofilm formation processes on different plastic substrates in the Northern Adriatic and 2) how the community composition differs between the incubated plastic polymers and the ambient seawater (ASW). We expect to observe differences in the

community composition between plastics exposed to solar radiation and held in the dark. The plastics incubated under dark conditions are expected to show a more diverse community composition and overall, a higher species richness.

## Material and Methods

### In situ incubation of plastic material

A frame was designed to incubate five common plastic types in Adriatic surface waters to study the influence of solar radiation on microbial colonization of plastics (Figure 1). The study was carried out approximately 1 km off the coast of Rovinj, Croatia, over a time period of 3 months (November 2016 - January 2017). Polyvinylchloride (PVC) pellets, containing two different plasticizers (DEHP and DINP), 4 $cm^2$-sized pieces of pristine polypropylene, low- and high-density polyethylene and glass slides were incubated in a floating structure under solar radiation and dark conditions. Pristine low-density polyethylene (LDPE), polypropylene (PP) as well as PVC were purchased from the Goodfellow Cambridge Ltd. Company, GRB. For high-density polyethylene (HDPE) samples, an empty and thoroughly rinsed chemical container (Sigma-Aldrich Co. LLC) was used. The glass pieces were cut from microscope slides (Carl Roth GmbH & Co. KG) and were used as an inert control to determine whether or not a specific plastic community establishes. Dark conditions were simulated using plumbing tubes, which allowed water exchange while keeping the solar radiation at a minimum inside the tube. The structure's frame was built of smaller plumbing tubes connected to four floats to ensure positive buoyancy. Inside the frame, four metal wires held the plastic pieces. The frame was linked to a main float with two 1.5m long tubes to keep artificial turbulence as small as possible. The structure was attached to a main buoy, which in turn was anchored to the seafloor. The shape of the structure and attachment was made so that the structure followed the direction of the currents, maximizing water circulation through the tubes. Prior to incubation, plastic pieces were rinsed three times with 70% ethanol and Milli-Q water before being fixed onto the wires using nylon strings. The plastics were sampled *in situ* after one week (November 2016), one month (December 2016) and two months (January 2017). At each sampling date, four pieces of each plastic type and glass slides were collected for analyses.

The pieces were sampled for DNA analyses and scanning electron microscopy (SEM). For community composition and DNA analyses, surface water was taken at each sampling day in two 2L polycarbonate bottles, which were rinsed three times with surface water immediately before sampling the plastics and the glass slides. Filtration was done in duplicate. Therefore, 2l seawater were filtered onto a 47mm 0.2µm Millipore (Merck KGaA) GTTP filters using a Millipore glass filtration unit. The 0.2µm-filtered seawater was kept for washing steps. Afterwards, the filters were folded and frozen at -80°C until further analysis. After sampling, the plastic and glass pieces were rinsed three times with the 0.2µm-filtered surface water to remove cells not firmly attached to the biofilm. The $4cm^2$ plastics were cut in smaller pieces using sterilized scissors to fit them in Eppendorf tubes. Pieces for DNA analysis were stored at -80°C after rinsing to prevent DNA from degradation. Samples for SEM were fixed in 4% glutaraldehyde (final concentration) for 10 min and then frozen at -80°C until further analyses.

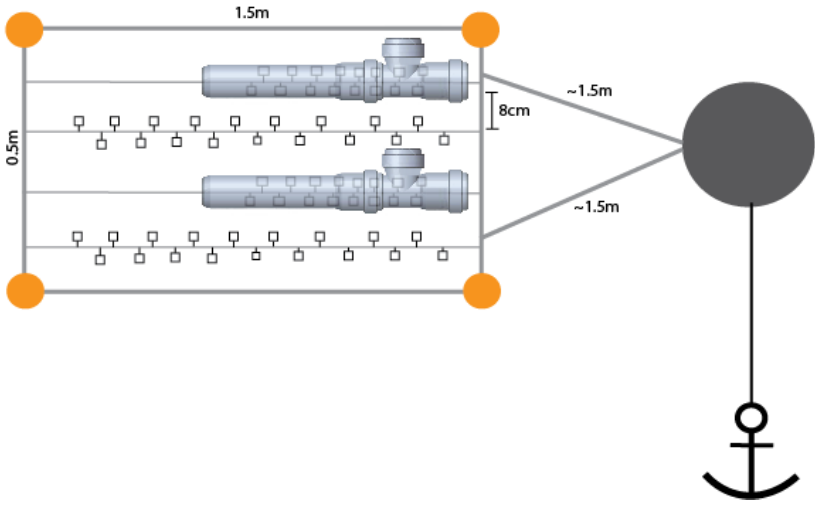


**Figure 1 -** Scheme of the incubation frame; orange dots = buoys, big grey dot = main float with anchor (from Langer, 2017)

### DNA extraction and amplification of the 16S rRNA gene

To investigate the microbial community composition of the biofilm, the DNA of the plastics was extracted and the 16S rRNA gene amplified for sequencing. Plastic samples from three time points were analyzed. The first time point was sampled after one week, the second after one month and last after two months of incubation. From the second time point only HDPE could be used, since a massive algal growth covered the other plastic types, changing the biofilm community. DNA was extracted from the five incubated plastic types

(including both conditions and all sampling times), glass and filters. The extraction was carried out using the Purgene Core kit A and a modified bead-beating protocol (see Supplementary Information). DNA extracts were stored frozen at -80°C for later analyses. The V4 region of the 16S rRNA gene was amplified using gene specific 341 ill NGS forward (5´-TCG TCG GCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3´) and 802 ill NGS (5´-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C-3´) reverse primers (Sigma-Aldrich Co. LLC.). The PCR was carried out in PCR tubes containing 50µl reaction mix (25µl Library Amplification Ready Mix 2xMM (Kapa Biosystems), 1µl of each primer (25µM) and 23µl sterile Sigma water plus the DNA template (volume depending on the DNA content - Supplementary Table 1). Sigma water was used as negative control. A Mastercycler Pro (Eppendorf AG, Germany) was set to 95°C for 3min initial denaturation, 20 cycles denaturation at 98°C, 20 cycles annealing at 56°C, 20 cycles elongation at 72°C and a final elongation for 5min at 72°C.

Products were separated by gel electrophoresis using a 2% agarose gel (1x TBE Buffer, 30min at 100V). Images of the SYBR® gold (Invitrogen™, Thermo Fisher Scientific) stained DNA were taken under UV light using a ChemiDoc Imaging system (Bio-Rad Laboratories Inc.) at 495 nm wavelength. PCR products were cleaned up in a 96-well magnetic plate using the Agencourt® AMPure® XP Purification kit and protocol (Beckman Coulter Life Sciences). DNA extraction yields were quantified using the Quant-iT™ PicoGreen® Assay following the protocol of the manufacturer (Invitrogen™, Thermo Fisher Scientific). Paired-end sequencing of the purified DNA extracts was performed using an Illumina MiSeq Sequencer and carried out by the Microsynth AG Company, Switzerland.

**Sequencing data Processing**

All processing steps of sequence data including merging paired reads, quality filtering (max. error 1 in 100 nucleotides), elimination of singletons, chimera filtering, mapping and OTU clustering were performed using the UPARSE OTU clustering Algorithm with USEARCH command lines[57]. For taxonomy assignment blastn and the SILVA_123_SSURef reference database were used (97% similarity between the sample and database sequences). Smaller changes in the USEARCH script, like the merging of taxonomy and OTU reads were written in Python programming language. OTUs with 5,000 reads or less were excluded from further analysis.

**Statistical Analysis**

All statistical tests and plots were conducted using R Studio Version 1.0.153 (RStudio, Inc. 2009-2017). The stacked bar plots were done using the relative abundances of the OTU reads and carried out with the ggplot2 package[58]. Diversity analysis and normalization (coverage-based rarefaction) of the samples was performed using the iNext package[59,60]. Analysis of Variance (ANOVA) or alternatively Kruskal-Wallis Rank test, if requirements were not met, was performed to test for significant differences between the diversity indices. Tukey HSD post hoc test was performed after the ANOVA to check for specific differences between the tested variables. The Box- and Dot-plot was plotted using the mean instead of the median and carried out using the ggplot2 package[58].

Diversity calculations were only done for the first and the last time point since the second time point only contained ambient seawater and HDPE and comparability was not met. To statistically test the difference in community composition between the samples, a PERMANOVA was performed for the first and the last time point using the relative abundances of the OTU reads, 999 permutations and the Bray-Curtis Dissimilarity. The second time point was not tested due to the deviating sample size as mentioned above. The Non-Metric Multidimensional Scaling (NMDS) was conducted to indicate overall patterns between the light- and dark-conditions using relative abundances of the OTU reads. Bray-Curtis Dissimilarity was used as a distance measure since it is most suitable for ecological data containing zero values. All calculations were carried out using the vegan Community Ecology package[61]. The Euler diagram was performed to illustrate the unique and shared OTUs using the eulerr package [62].

**Scanning Electron Microscopy (SEM)**

To check the biofilm structure, SEM samples were dehydrated in a step-wise series of baths of 30%, 50%, 70%, 80%, 90%, 95% ethanol each for 10min and three times in 100% absolute ethanol for 10min. The dehydrated samples were $CO_2$ critical point dried with a CPD 300 auto critical point dryer (Leica Microsystems). The dried pieces were gold coated using a JFC-2300HR sputter coater (JEOL Ltd.) for 80s. Pictures were taken with a JEOL JSM-IT300 scanning electron microscope at a magnitude of 450x at 15kV-20kV.

## Results

### Microbial biofilm formation and community composition

The microbial community composition of the plastic associated biofilm was compared to the community on glass and in the ambient seawater (ASW). After a week of incubation, a diverse biofilm on the plastic surfaces could be observed (Figure 2). In the initial phase of the biofilm formation, the most abundant phyla observed in all samples and under both, light and dark conditions were Proteobacteria, Cyanobacteria, Bacteroidetes and Planctomycetes. Cyanobacteria were dominating under light conditions, while Bacteroidetes, Planctomycetes and Proteobacteria were more dominant in the dark treatment throughout the incubation period. The difference in microbial community composition between the substrates (plastics and glass) and the ASW was clearly visible during the entire incubation period (Figure 2). No differences, however, were observed in the community composition between the glass and the plastic types from the beginning of the experiment onwards under both conditions. The PVCs showed a distinct and variable community composition, differing clearly from all other incubated substrates in both treatments and throughout the entire incubation period (Figure 2). In the initial incubation phase, Proteobacteria was the most abundant phylum in all samples under dark conditions and in the PVC samples under light conditions, with a relative abundance of up to 90% in the PVCs and 40% - 60% in the other samples (Figure 2).

Under the light condition, Cyanobacteria rather than Proteobacteria dominated the bacterial community of the non-PVC plastics, with a relative abundance of up to 75%. The biofilm communities of the two PVC types in the light treatment showed an increase in the relative abundance of Cyanobacteria towards the end of the incubation. The community composition of the HDPE under light conditions after one month of incubation differed clearly from that of the HDPE samples of the other two time points. Furthermore, the community composition of the HDPE in the light treatment after one month of incubation showed a higher similarity to the community composition under dark conditions of the same time point than to the biofilm community of the HDPE in the light treatment of the initial and final time point (Figure 2).

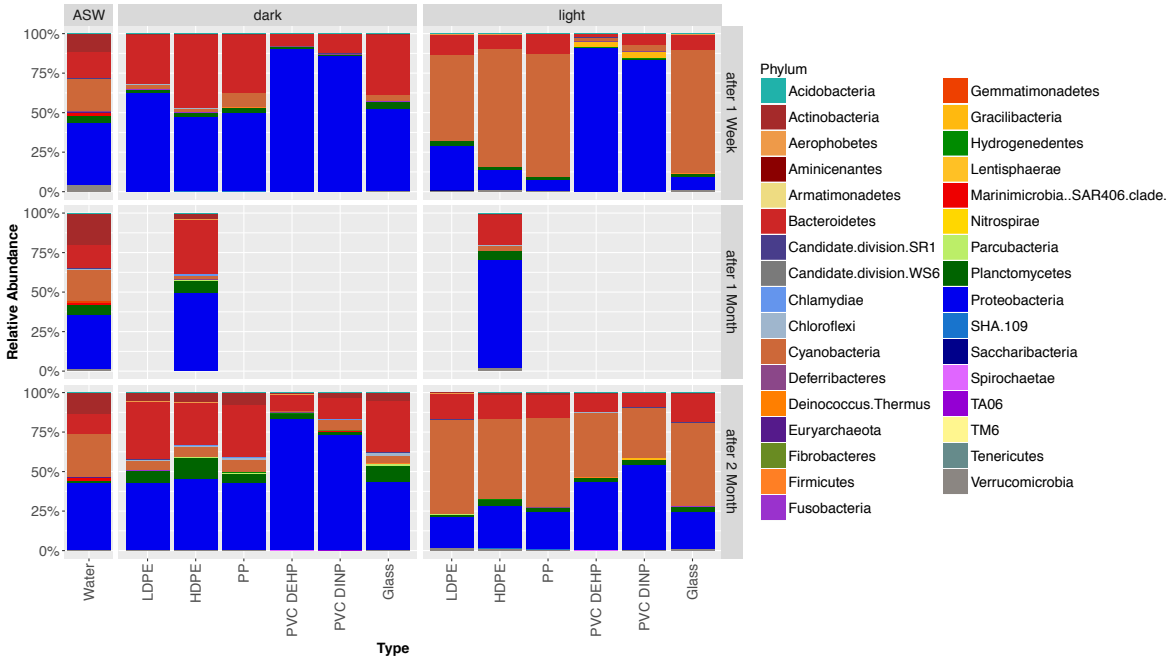


**Figure 2 -** Stacked bar plot illustrating the relative abundances of all phyla shown for each plastic type/glass, light and dark treatment (column) and time point (row).

The relative abundances of the 29 most abundant families for each sample, time point and condition (light or dark) are shown in Figure 3. Under light conditions a large fraction of the relative abundance was assigned to "unknown family" and was therefore plotted separately to present a clearer pattern of the remaining families. The "unknown family" category was composed mainly of unknown and uncultured Cyanobacteria (Supplementary Figure 2). As shown in Figure 3, the community composition of the ASW was different from that on the plastics and the glass slides throughout the incubation period. Although the light and dark conditions differed in their community composition (Figure 3), the variations were not as clear as on the phylum level illustrated in Figure 2. Furthermore, no similarities in the community composition between the glass and plastics were observed throughout the incubation period as already described above (Figure 2, 3). The PVCs under light and dark conditions showed a unique community composition compared to the other substrates at both time points, but were particularly distinct after one week of incubation (Figure 3).

The most abundant families were the Flavobacteriaceae, Rhodobacteraceae, Alteromonadaceae and the Hyphomonadaceae. At each time point, one of the most abundant families was the family Flavobacteriaceae, occurring in every sample, but in lowest abundances on the PVCs at the beginning of the incubation (Figure 3). Alteromonadaceae occurred predominantly in the initial phase of the incubation under light conditions,

exhibiting the highest relative abundance in the biofilm of the PVCs reaching a relative abundance up to 85% (Figure 3). The family Rhodobacteraceae had the highest relative abundance in the HDPE sample after one month of incubation under light conditions, making up to 50% (Figure 3). Figure 3 also shows the relative abundance of the family Hyphomonadaceae occurring in the biofilm of all samples under both conditions in the final phase of incubation, reaching the highest relative abundance on the PVC samples after two months of incubation (Figure 3).

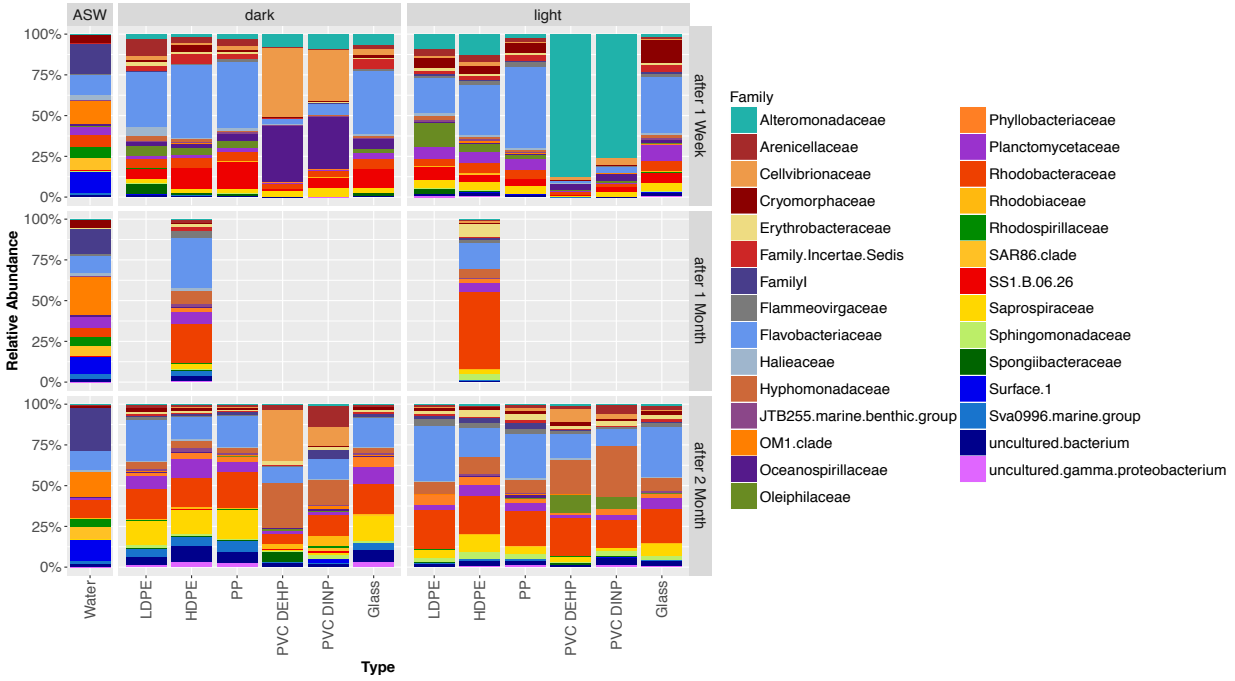


**Figure 3 -** Stacked bar plot showing the relative abundances of the 29 most abundant families for each sample, condition (column) and time point (row).

Hydrocarbon-degrading taxa were found in all the samples. These taxa were mainly present on the plastics and glass slides and only rarely in the ASW. Since not all detected hydrocarbon-degrading taxa were members of the 30 most abundant families they are not depicted in Figure 3. The family Alteromonadaceae entails the known hydrocarbon-degrading genera *Alteromonas* and *Marinobacter* and was dominating the biofilm on the PVCs after one week of incubation under light conditions (Figure 3). The Flavobacteriaceae were detected in all samples, time points and under both conditions, showing a high relative abundance at the beginning of the incubation and the highest relative abundance in the polypropylene biofilm under light conditions, contributing up to 60% of the total abundance. This family contains the known hydrocarbon-degrading genera *Formosa* and *Winogradskyella* (Figure 3). Another abundant family containing hydrocarbon-degrading

genera is the Hyphomonadaceae, which occurred in every sample at the end of the incubation under light as well as dark conditions, showing the highest relative abundance in the biofilm of PVC. A known hydrocarbon-degrading genus involved in oil-degradation is the genus *Oceaniserpentilla,* belonging to the family Oceanospirillaceae and exhibiting the highest relative abundance of nearly 40% on the PVC after one week of incubation under dark conditions (Figure 3). *Oleiphilus,* belonging to the family Oleiphilaceae, is also known to be an oil-degrading genus. *Oleiphilus* occurred in all samples at the initial time point, in both conditions and after two months of incubation under both conditions on PVC in low relative abundances (Figure 3). The hydrocarbon-degrading genera *Labrenzia* and *Roseobacter* are a member of the family Rhodobacteraceae, which was present in all samples including ASW, time points and conditions. Both genera exhibited the highest relative abundance on HDPE after one month of incubation (Figure 3). Other hydrocarbon-degrading taxa, not abundant enough to be depicted in Figure 3, were the family Alcanivoraceae with the genus *Alcanivorax*, which was more abundant at the beginning of the incubation under dark than under light conditions and showed the highest relative abundance in the LDPE. Erythrobacteraceae with the genus *Erythrobacter* was abundant throughout all samples but peaked in relative abundance after one month of incubation on the HDPE under light conditions. The family Pseudoalteromonadaceae with the genus *Pseudoalteromonas* was abundant at the beginning of the incubations under dark conditions on all substrates. Other known hydrocarbon-degrading Bacteria present, although in very low abundances, were Bartonellaceae with the genus *Bartonella* and Coxiellaceae with the genus *Coxiella.*

A clear clustering in bacterial community composition of the biofilms exposed to light and dark conditions and incubation times was detectable (Figure 4, Supplementary Figure 1). The ASW grouped in a distinct cluster, containing all three incubation times and showing no similarities with the biofilm community growing on the solid substrates (Figure 4). The biofilm community on the glass slide of the initial and final time point clustered together with the biofilm community of the plastic types in the respective treatments and time points. The community of the PVCs of the initial incubation time in both treatments and the PVCs of the final time point of the dark treatment formed distinct groups distant from the biofilm community of the other substrates in the respective treatments (Figure 4). The composition of the microbial communities growing on the PVCs at the final incubation time under light condition, however, showed similarities with the community composition of the

other substrates under the respective conditions (Figure 4). The biofilm communities of both HDPEs from the mid experiment time point clustered together with those of the non-PVC substrates at the final time point in the dark treatment.

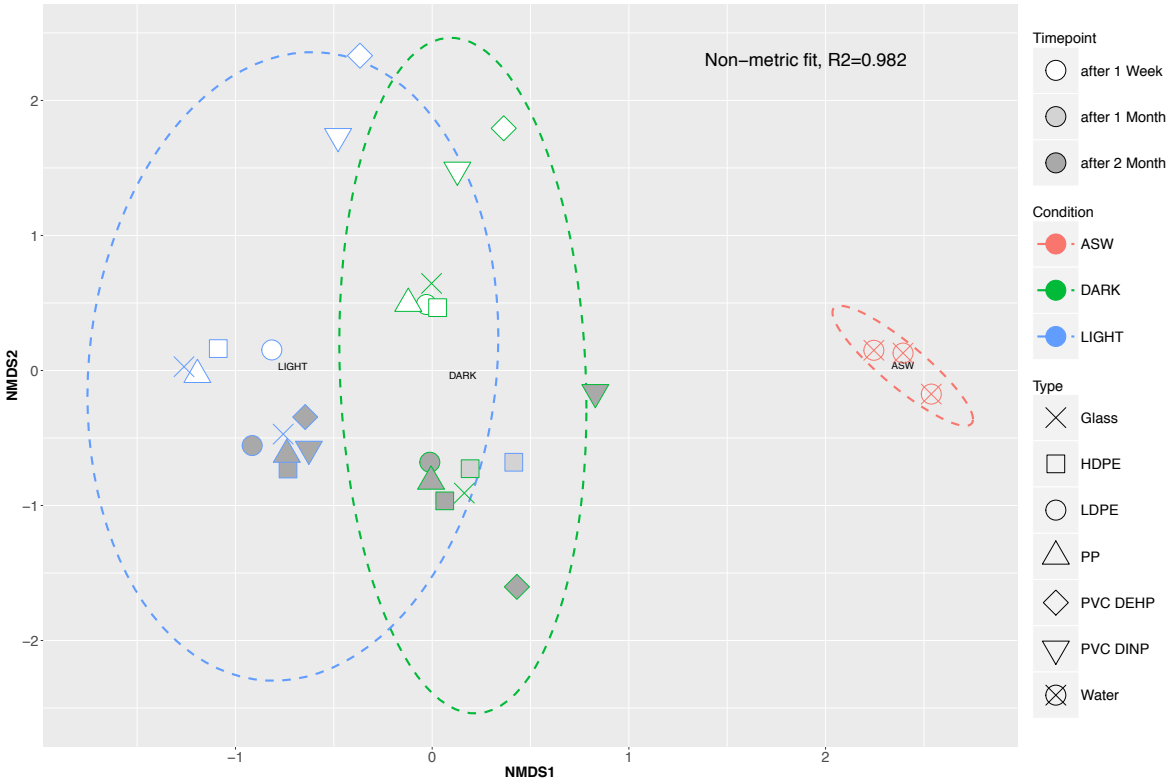


**Figure 4 -** Non-metric Multidimensional Scaling with relative abundances of OTU reads and distances between condition groups and substrate types calculated with Bray-Curtis dissimilarity.

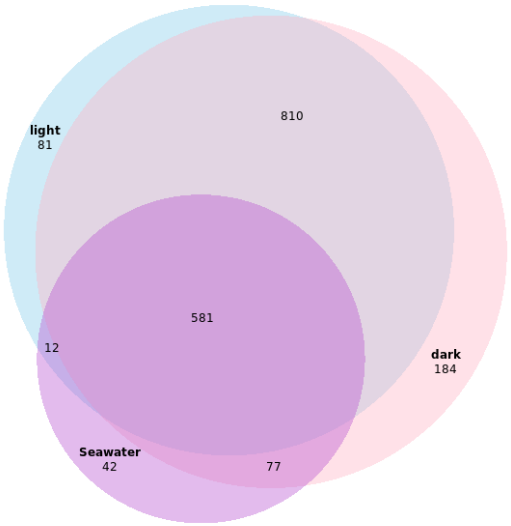


**Figure 5 -** Euler Diagram showing unique and shared OTUs for the light and dark conditions on all substrates and the ASW. Numbers represent number of OTUs and colours depict the light /dark condition and the ASW.

Figure 5 depicts the distribution of unique and shared OTUs between the light and dark conditions of all substrate types and of the ASW throughout the incubation period. The light and dark treatment shared the majority of OTUs. The ASW shared some OTUs with the substrates but the majority was only found on plastics and glass. The dark condition exhibited the most unique OTUs with more than double the amount compared to light condition. Significant differences in community composition between the light and dark conditions or substrate types illustrated in Figures 2-4, were found using a PERMANOVA (Table 1). Also the community composition of the PVCs and the other substrate types was significantly different (Table 1).

**Table 1 –** Significance levels of the bacterial community composition using PERMANOVA between the light and dark condition and the substrate types of the first and last time point. PERMANOVA was performed using the relative abundances of the OTU reads with 999 permutations and the Bray-Curtis Dissimilarity. Only the community composition of the solid substrates was used in each time point, ASW was not included. Significance levels: p<0.001="***", p<0.01="**", p<0.05="*".

| | **After one week incubation** | **After two month incubation** |
|---|---|---|
| **Light vs. Dark condition** | Pr (>F) = 0.003** | Pr (>F) = 0.003** |
| **PVCs vs. other Substrate types** | Pr (>F) = 0.028* | Pr (>F) = 0.035* |

**Diversity Indices**

The diversity indices were calculated to further support the already illustrated pattern shown in Figures 2-4. Figure 6 shows the Shannon Index/species richness of the light and dark condition for the beginning and end of the incubation period, thus after one week and two months of incubation. The statistical tests of the diversity indices confirmed a substantial difference between the light and the dark treatment. The dark condition showed, for both time points, a significantly ($p<0.05$) higher mean Shannon Index compared to the light condition. In the final time point the species richness showed a significantly ($p<0.05$) higher mean in the dark condition compared to the initial time point, where no significant difference between the light and dark treatment was depicted (Figure 6).

In Figure 7, the species richness of the six incubated substrates (LDPE, HDPE, PP, PVC DEHP/DINP, glass) is illustrated for the initial and final time point of the incubation. The PVC DEHP depicted a significant ($p<0.05$) lower mean species richness in the initial incubation time point compared to the other substrates. The results of the Tukey HSD post-hoc test (Supplementary Table 2) showed a significant difference in the mean species richness

between PVC DEHP and polypropylene (PP). In the final time point of the incubation this pattern was not observed. Overall, the species richness was higher in the final time point compared to the beginning of the incubation. However, the species richness of the PVC DEHP increased towards the end of the incubation period, when no significant differences in species richness between PVCs and the other substrates were detectable any more (Figure 7).

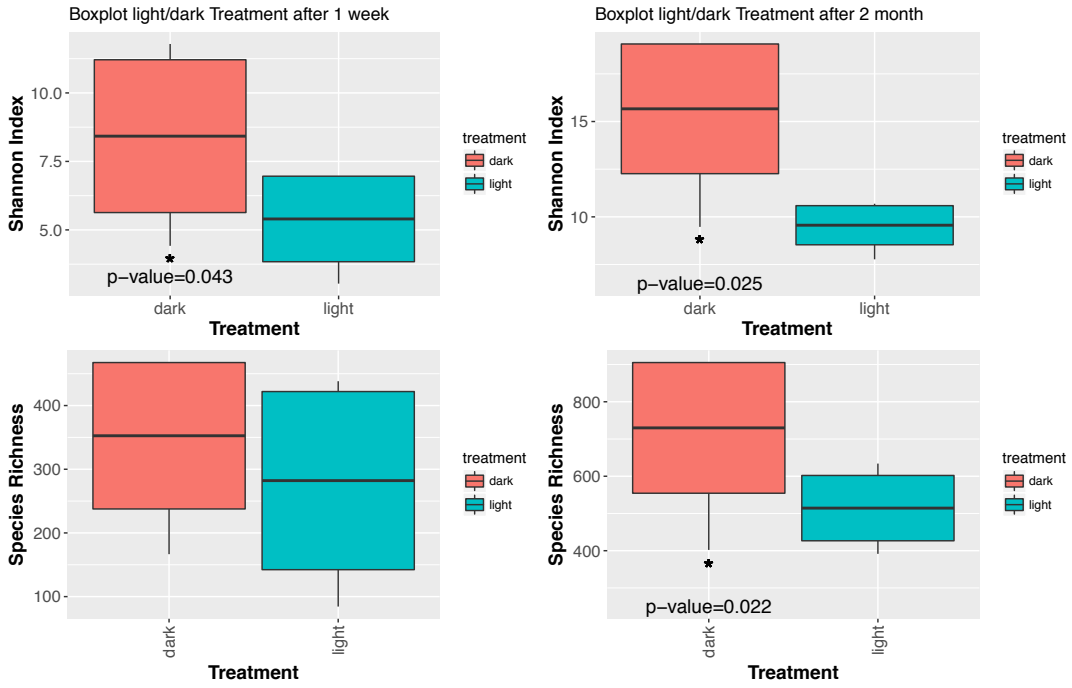


**Figure 6 -** Diversity Indices showing the differences between incubated substrates in the light and dark conditions for the initial and the final time point. Significance level: p<0.001="***", p<0.01="**", p< 0.05="*"; significance was tested with an ANOVA or a Kruskal-Wallis rank test (Shannon Index after two months/final time point).

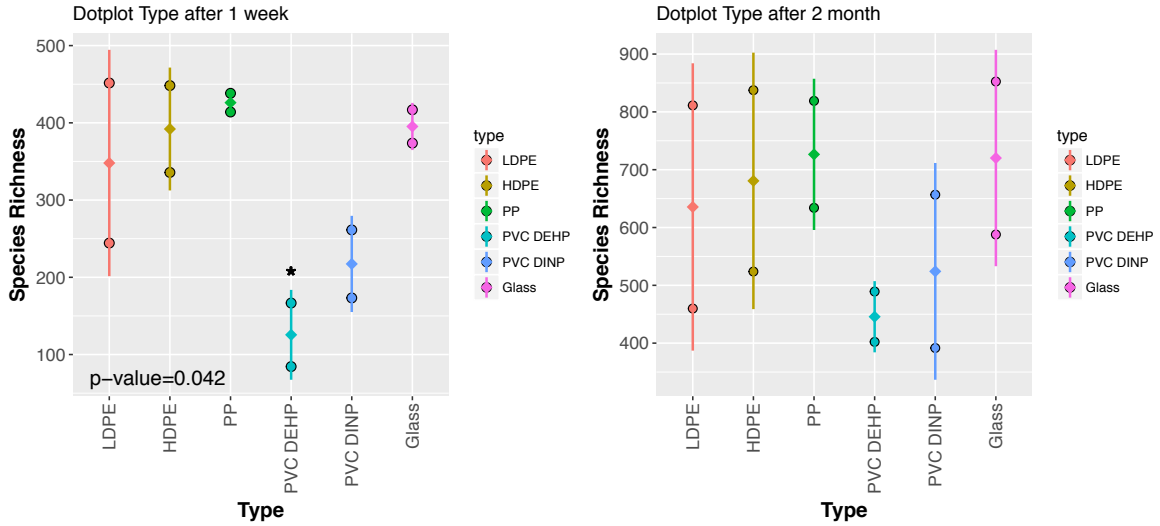


**Figure 7 –** Bacterial species richness of the incubated substrate types for the initial and the final time point. Dots are representing the sample of each treatment, for each substrate type, respectively. The diamond shape represents the arithmetic mean +/- standard deviation. Significance levels: p<0.001="***", p<0.01="**", p<0.05="*"; significance was calculated with an ANOVA.

**Scanning electron Microscopy (SEM)**

The electron microscopy images depicted differences in biofilm structure and coverage between the substrate types, time points and light and dark conditions. The HDPE had a biofilm not completely covering its surface in the initial phase of the incubation. After one month of incubation, a patchy biofilm was visible under both conditions (Figure 8). Under light conditions, at the final time point of the incubation diatoms were abundant. The surface of the HDPE was very rough compared to the smooth surface of glass or polypropylene and showed similarities to the rough surface of PVC DEHP (Figure 8-10 and Supplementary Figure 3). The SEM pictures of the HDPE showed a successional biofilm formation, with no or little coverage at the beginning of the biofilm and a thicker, but still patchy biofilm at the end of the incubation.

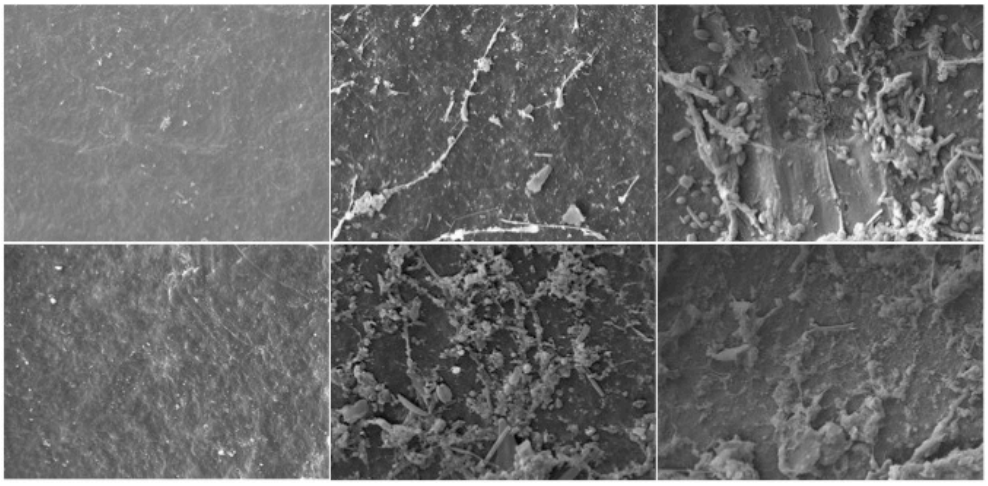

**Figure 8 -** SEM pictures showing HDPE samples throughout the entire incubation time. The upper panels show the light and the lower panels the dark conditions. Columns from left to right: HDPE after one week, HDPE after one month, HDPE after two months of incubation.

The glass surface (Figure 9) showed a very smooth surface structure. At the beginning of the incubation no covering biofilm was visible, only very thin patches. With increasing incubation time, the biofilm became more diverse and thicker patches were formed. Under light conditions, diatoms were occurring becoming more abundant over time. At the final time point, big patches of the glass were completely covered with a thick layer of diatoms (Figure 9, upper right corner). Figure 10 shows the SEM pictures of the PVC DEHP at the initial and final time point under light and dark conditions. The surface of the PVC exhibited a wavy structure, similar to the surface of the HDPE (Figure 8). Already after one week of incubation a thick and patchy biofilm layer had formed.

The biofilm coverage increased slightly over the incubation time but the thickness and patchiness did not visibly change. Compared to the other plastics, PVC had the thickest biofilm and was formed the fastest.

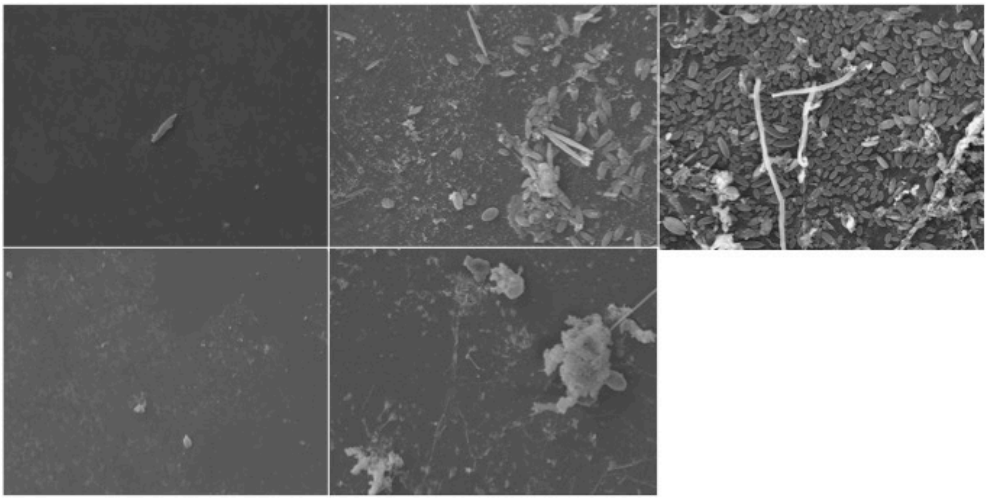

**Figure 9 -** SEM pictures showing glass slides with biofilm development over the incubation period. The upper panels show the light and the lower panels the dark conditions. Columns from left to right: glass after one week, glass after two months of incubation. The picture in the upper right corner also shows glass after two months under light conditions.

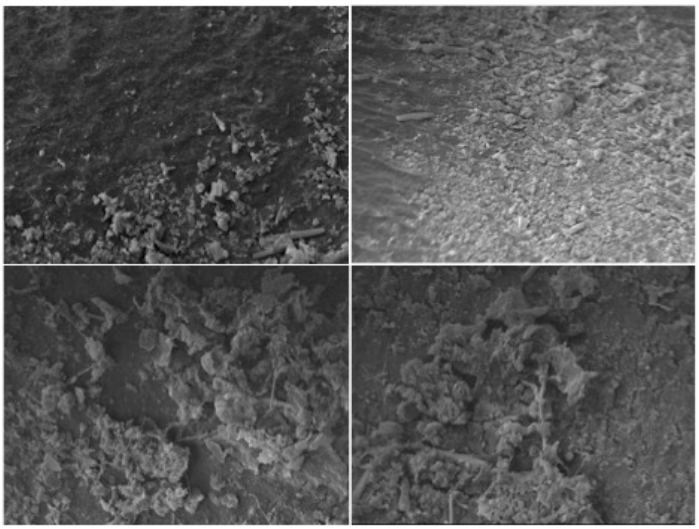

**Figure 10 -** SEM pictures showing PVC DEHP biofilm development over the incubation time. The upper panels show the light and lower panels the dark conditions. Columns from left to right: PVC DEHP after one week, PVC DEHP after two months of incubation.

## Discussion

### Particle-attached vs. free-living microbial communities

This study was conducted to examine the effects of natural sunlight on microbial biofilm development on different plastic substrates. 16S rRNA amplicon sequencing was performed to investigate differences in microbial community composition, while SEM was used to compare the surface properties and biofilm matrices of different plastic surfaces during the course of the incubation in the surface waters of the northern Adriatic Sea.

The genetic analysis showed a clear difference between the light and dark treatments and the plastic types. Comparisons between the incubated substrates and the ASW revealed a clear difference in microbial community composition, while there was no obvious difference between the incubated non-PVC samples and the glass. The PVC samples, however, showed clear differences to all other samples. This disparity between free-living and plastics-associated microbial communities was observed before by several studies [25,30]. The absence of a distinguishable difference between plastic- and glass-associated communities was only observed in one previous study [40]. This implies that the driving force of biofilm development is rather the availability of substrate than the actual substrate type. Since this study was conducted over a 2-months period, a prediction on how the community composition might have changed after a longer incubation time is not possible. Biofilm formations are successional processes and the climax state of the biofilm community was probably not reached at the end of the two months incubation period. It could be possible that the initial microbial colonizers were not specialized when settling and therefore just picked any available substrate, which would explain why there were no distinct differences between the microbial community composition on the glass and the plastics.

### Plastic Types

Distinct differences were observed, however, between some of the incubated plastic types, suggesting that the substrate types or rather the surface properties could indeed play a key role in the establishment of a biofilm. The difference in community composition between the PVCs and the other substrates was significant (Table 1). These results indicate that the microbial biofilm community composition of the PVCs was different from that of the other plastic substrates as shown in Figure 2-4. At the start of the incubation both PVC types exhibited a similar community composition, but different from that on glass or the other

types of plastics (Figure 2, 3). This pattern was also shown on the SEM pictures (Figure 9). The PVC DEHP samples depicted a diverse and thick biofilm already after one week of incubation. It differed clearly from the biofilm on glass and HDPE (Figure 8, 10), indicating the unique pattern in community composition shown in Figures 2-4. One possible explanation could be the difference in softness of the material or the surface roughness.

PVC is, compared to the other plastic material, much softer due to the high concentration of added plasticizers during the production process[6,7]. Even though the influence of plastic softness on biofilm formation has never been shown, it is known for other surfaces (e.g. cardboard, leaves, metal, tiles, plastic and aluminum) that differences in the hardness of the material result in different microbial community composition[63]. This difference and possible influence of softness on biofilm establishment can be seen when comparing the biofilm formation on a soft material like PVC, hard plastic like HDPE and even harder material like glass in the SEM pictures (Figure 8-10). Another variable affecting the biofilm community could be the difference in surface roughness. It has been shown that surface properties of different materials influence biofilm establishment and settlement processes [64]. The surface roughness of the PVCs was visible and clearly distinguishable from the other incubated plastics, showing a clear wavy surface in contrast to the completely smooth surfaces of the polypropylene and glass (Supplementary figure 3, 9). The HDPE material also showed a wavy surface under the SEM (Figure 8). This is surprising since the community composition of the HDPE was not as distinct as the community composition of the PVC, even though the surface properties were similar. The HDPE exposed to solar radiation showed a different microbial community composition after one month of incubation as compared to the other samples under light conditions but apart from that, there was no significant difference. This would indicate that the surface properties are not the only factor influencing biofilm establishment. Furthermore the surface roughness fails to explain the results shown in Figure 7, indicating a significantly lower mean species richness on PVC DEHP in the initial time point compared to the other plastic types and showing no difference in species richness at the end of the incubation period, at which the species richness is overall higher than in the initial time point. If microbes would favor surface roughness to settle, then more would be capable to attach to the surface at the beginning of the incubation, resulting in higher species richness on the PVC in the initial time point. However instead of showing a higher species richness, the results depicted lower mean

species richness on the PVC DEHP compared to the other substrates in the initial time point (Figure 7), failing to support the "roughness hypothesis".

Nevertheless PVC plasticizers (DEHP and DINP) might also play a role in shaping the biofilm composition. It was indicated that these plasticizers can have carcinogenic and/or endocrine disrupting effects on fish and other vertebrates, however, the influence on microbial metabolism and activity is largely unknown[8]. These additives can leach into the surrounding environment since they are not part of the chemical structure of the plastic[6,7,65]. This could be a possible explanation for the lower mean species richness observed in the PVC DEHP at the beginning of the experiment (Figure 7). Perhaps the leaching plasticizers are harmful to some microbes, preventing them from settling, while enabling some more resistant species to thrive, thus leading to lower species richness on the PVC DEHP compared to the other substrates (Figure 7). The lack of a significant difference in species richness between PVC DEHP and the other substrates at the end of the incubation period might be due to the fact, that the biofilm gets thicker over time and consequently less plasticizers are leaching out, resulting in more microbes settling and a higher species richness in the final time point compared to the beginning of the incubation. The phthalate and additive DEHP is known to be more toxic than its substitute DINP, which is considered to be not hazardous and a smaller risk to the environment[6]. This would explain the significant lower species richness of only PVC DEHP but not PVC DINP (Figure 7). However, further investigations are needed to clarify the possible effects of plasticizers on microbial biofilm formation and composition and to study additive leaching over time.

**Influence of solar radiation on microbial community composition of plastics**

A significant difference between light and the dark conditions was observed. A condition-specific microbial community composition was obtained (Figure 2) with Cyanobacteria dominant under light conditions. This high abundance of Cyanobacteria is due to their photoautotrophic nature contributing a major part to the photoautotrophic biomass in the Mediterranean Sea[66]. Cyanobacteria were also an important biofilm component on plastics in other studies[25,29]. The SEM pictures of the glass surface after two months of incubation (Figure 9) under light conditions depicted a high abundance of diatoms, which could not be seen in this abundance on any other material used in this study.

Alteromonadaceae were the dominant heterotrophic microbial family under light conditions after one week of incubation (Figure 3), known to be highly resistant to solar radiation[51]. Furthermore, Alteromonadaceae and other members of Gammaproteobacteria are known to be pioneers in microbial biofilm formation on artificial substrates, while Alphaproteobacteria settle at later stages of the colonization succession[67,68]. This explains the high abundance of *Alteromonas* at the initial phase and their absence towards the end of the incubation. Overall a higher diversity was found in the biofilms held in the dark (Table 1, Figure 4, 6). The disparity in diversity between the light and the dark condition reflects the influence solar radiation has on the biofilm establishment and also the community composition. It was shown in several studies that solar radiation damages the DNA of microorganisms and inhibits bacterial activity[47,52]. Another factor that should be considered is the interspecific difference in sensitivity to solar radiation between microbial groups, which might also influence biofilm establishment, community composition and diversity by supporting the more resistant and inhibiting the solar radiation-sensitive taxa [56].

**Putative hydrocarbon degrading microbes on plastic surfaces**

Different studies revealed that many genera of the phylum Proteobacteria, class Alpha- and Gamma-Proteobacteria (*Alcanivorax, Alteromonas, Pseudomonas, Marinobacter*, etc.) belong to bacterial communities that are involved in hydrocarbon-degradation (e.g. crude oil) processes[38,49,69]. Not all families containing hydrocarbon-degrading genera are shown in Figure 3. In our study, the genus *Alteromonas*, which is one of the known hydrocarbon-degrading genera of the family Alteromonadaceae, was highly abundant in the initial phase of the incubations under both, light and dark conditions, however, particularly in the PVCs under light condition.

Specific genera are more abundant under light conditions (*Alteromonas, Marinobacter, Bartonella*, etc.), while others are dominant in the dark like *Thalassobius, Winogradskyella, Alcanivorax,* etc.[49]. In this experiment, the same potential oil-/hydrocarbon-degrading genera were found, but no clear affiliation to a particular condition could be deciphered. *Oceaniserpentilla* was the sole genus occurring only under dark conditions after one week of incubation. *Oceaniserpentilla* was also found on plastics in the study by Zettler et al. (2013) and was abundant after the Deep Water Horizon oil spill, indicating that it belongs to the oil-degrading community[25,69]. All other genera could be

detected under both conditions, although the abundance was often higher in one of them like, for example, *Alcanivorax,* family Alcanivoraceae, which was more abundant under dark conditions. The family Hyphomonadacea, described to be able to degrade hydrocarbons, was found on plastic biofilms [25]. These hydrocarbon-degrading bacteria were more abundant at the initial than at the later phase of incubation. This result suggests that the settlement of these genera was substrate-induced rather than biofilm-induced. Substrate-induced biofilm formation implies that the substrate itself played a key role in the settlement process and eventually indicates the possibility that the plastics attracted microbes, as it might serve as a carbon source. If the settlement would be biofilm-induced the microorganisms would be attracted by the biofilm polysaccharide matrix on the plastics instead, which can be used as carbon- or nutrient-source [70].

## Conclusion

In this study the influence of solar radiation on the biofilm establishment on different plastic substrates was investigated in the marine environment. The incubated plastics were quickly covered with a diverse biofilm, showing a distinct community composition that clearly differed from that of the ambient seawater. The community composition on the glass samples, however, did not differ from the biofilm community on the non-PVC plastic substrates, indicating that the driving force of biofilm development is rather the availability of substrate, than the type of substrate for the majority of the colonizing microbes. A possible factor influencing biofilm establishment and development are the surface properties, such as roughness and softness, as indicated by the differences in community composition between the PVC samples and the other substrates. Plasticizers could also play a role in biofilm establishment, but how and if they affect microbial communities is not sufficiently studied yet and further investigations are needed. A clear and significant difference in terms of community composition between the dark and light treatment could be shown throughout this study. Phototrophic taxa were dominating in the light condition. The overall diversity was higher under dark conditions throughout the incubation period. Different hydrocarbon-degrading taxa were found in the biofilm at the initial phase of the incubation of plastics in the surface waters suggesting the potential of plastic being used as a carbon source.

However, no degrading processes or indications of plastic utilization as a nutritional source were observed. The potential of these taxa to degrade plastics needs to be studied further.

Taken together, this study showed that solar radiation influences microbial biofilm establishment on plastics. Hydrocarbon degrading microorganisms were present, even though no actual degrading processes could be observed. Differences between the incubated substrates and the ambient seawater in terms of community composition could be shown, as well as differences in biofilm community composition between the PVCs and the other substrates. Future studies should focus on the effects of solar radiation on biofilm establishment, the role surface properties play in biofilm formation on artificial surfaces and how plasticizers might influence or affect microbial biofilm communities**.**

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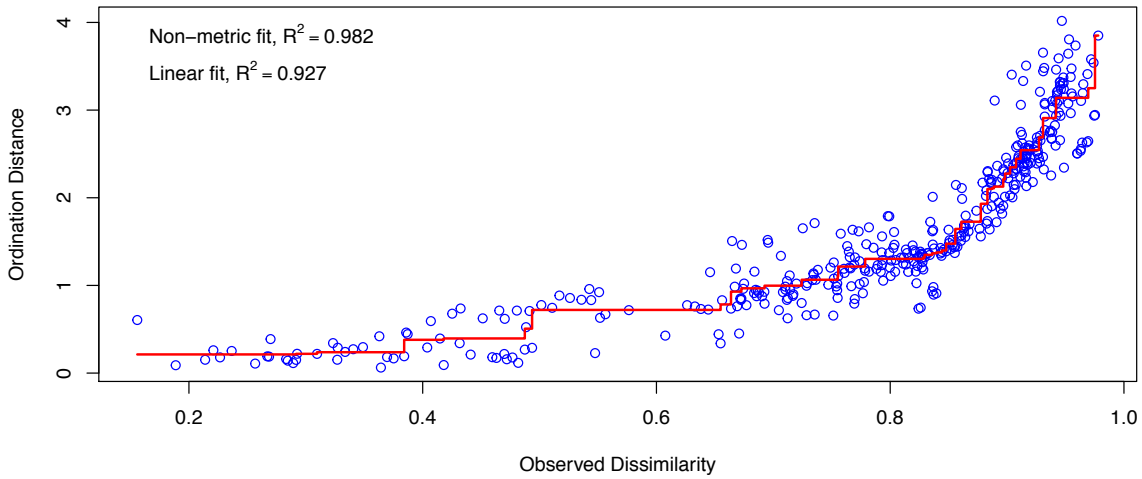
## Supplementary Information

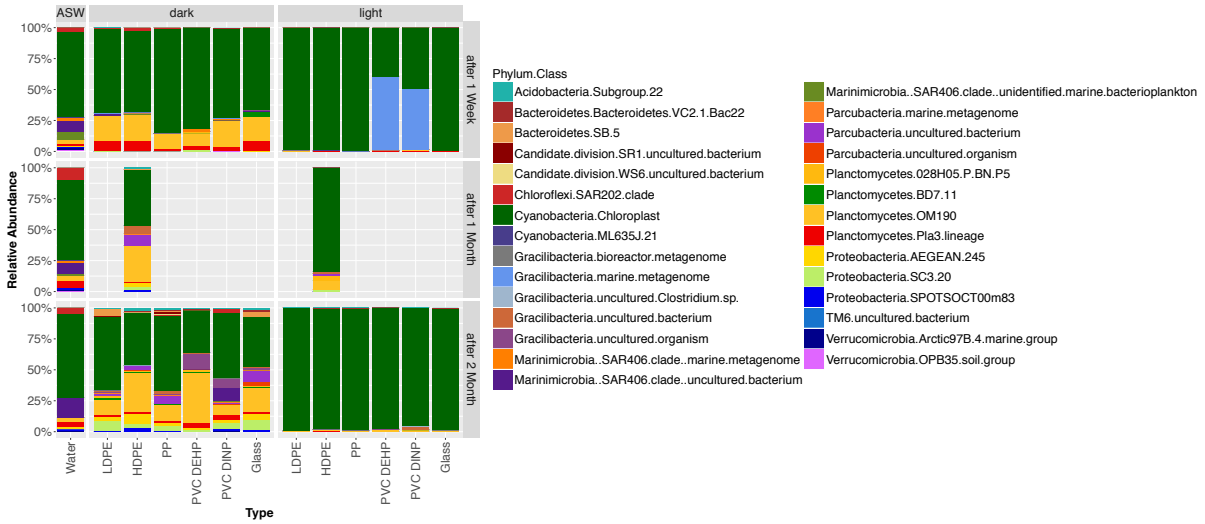



**Supplementary Figure 1 -** Stress plot showing the "goodness of fit" of the data in the NMDS plot. The closer the $R^2$ is to 1, the better is the goodness of fit and the more precise the groupings are plotted.

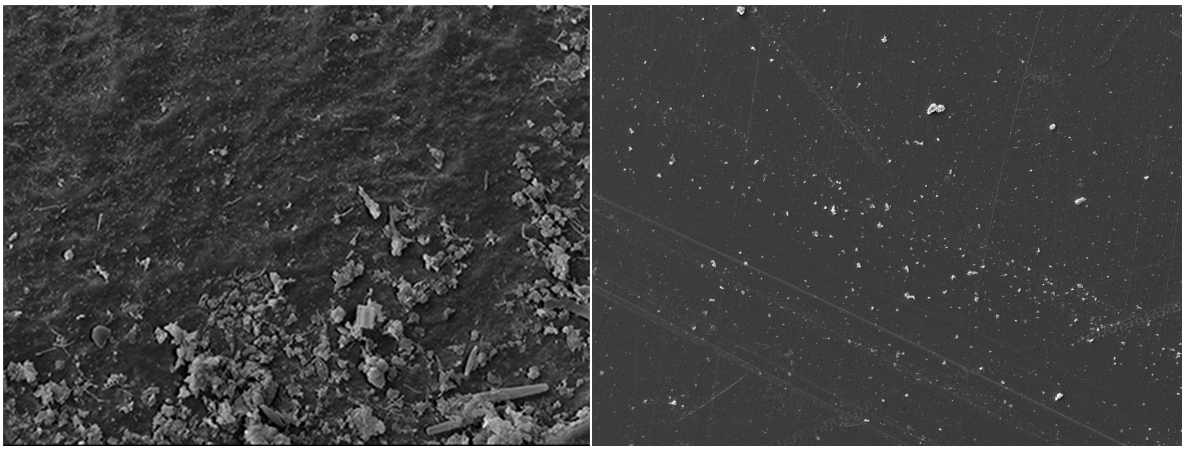
**Supplementary Table 1 -** DNA concentrations determined via Quant-iT™ PicoGreen® Assay measurements of the incubated samples, including surface water DNA.

| Sample N° after 1 week Inc. | Conc. (ng/µl) | Sample N° after 1 Month Inc. | Conc. (ng/µl) | Sample N° after 2 Month Inc. | Conc. (ng/µl) |
|---|---|---|---|---|---|
| LDPE light * | 0.01 | | | LDPE light | 0.41 |
| HDPE light | 0.02 | HDPE light | -0.05 | HDPE light * | 0.04 |
| PP light | 0.05 | | | PP light * | 0.14 |
| PVC DEHP light | 3.33 | | | PVC DEHP light * | 0.09 |
| PVC DINP light | 1.67 | | | PVC DINP light * | 0.05 |
| Glass light | 0.00 | | | Glass light | 0.17 |
| LDPE dark | 0.01 | | | LDPE dark | 0.11 |
| HDPE dark | 0.03 | HDPE dark | 0.14 | HDPE dark | 0.18 |
| PP dark | -0.02 | | | PP dark | 0.20 |
| PVC DEHP dark | 0.81 | | | PVC DEHP dark | 0.31 |
| PVC DINP dark | 0.26 | | | PVC DINP dark | 0.06 |
| Glass dark | -0.06 | | | Glass dark | 0.09 |
| ASW Nov 2016 * | 0.12 | ASW Dec 2016 * | 0.06 | ASW Jan 2017 * | 2.07 |

* samples that deviated from the normal DNA template volume of 1 µl for the 16S rRNA amplification (LDPE light = 2µl template, ASW samples and samples after 2 month of incubation 1µl of 1:10 diluted template)




**Supplementary Figure 2 -** Stacked Plot of the "unknown families" found under the 30 most abundant families per sample, conditions and time point. This "unknown family" was plotted separately since it made up 70% of the relative abundance under light conditions and thus, changed the main pattern between the samples.



**Supplementary Figure 3 -** Scanning electron microscopy pictures. Left: PVC DEHP light, after one week of incubation; Right: PP dark, after one week of incubation. Both pictures were taken at 450x magnitude. The PVC picture (left) shows clear waves in the upper left corner, while the surface of the PP (right) is completely smooth

## Modified bead-beating protocol

Samples were incubated in 500µl of lysis solution (kit) and 10µl lysozyme (1000u $ml^{-1}$) at 37°C for 30min. Afterwards 5µl proteinase k (kit) was added and mixed by inverting 25 times. Approximately 0.325g of beads were added per tube before bead beating two times for 45s (FastPrep™, MP Biomedicals, USA). After incubating for another 30min at 55°C, 4µl RNAse (kit) was added and mixed by inverting 50 times. Following a 30min incubation at 37°C and 5min on ice, 250µl of protein precipitation solution (kit) was added to each tube and the tubes vortexed at high speed for 20s.

After centrifuging the tubes for 3min at 14,000x*g*, the supernatant was transferred to a fresh tube containing 750µl 100% isopropanol and inverted 50 times. Following another centrifuging step at 14000x*g* for 5min, the supernatant was discarded and tubes were drained from remaining isopropanol. As a washing step, 750µl 70% ethanol was added and the tubes were inverted several times. After centrifugation for an additional 3min, the supernatant was discarded again and tubes were drained and air-dried. The remaining DNA extract was re-suspended in 40µl hydration solution (kit) and incubated at 65°C for 45min to completely dissolve. The DNA extract was then frozen at -80°C until further analysis.

**Supplementary Table 2 -** Results of the Tukey HSD Test - multiple comparisons of means between substrate types of the initial time point (after one week incubation). Table showing the differences between group means, the lower and upper confidence intervall bounds and the adjusted p-value, all calculated with a 95% confidence intervall.

| | diff | lwr | upr | p adj |
|---|---|---|---|---|
| **HDPE-LDPE** | 44.0365 | -265.49111 | 353.564109 | 0.9899674 |
| **PP-LDPE** | 78.153 | -231.37461 | 387.680609 | 0.9007365 |
| **PVC DEHP-LDPE** | -222.439 | -531.96661 | 87.088609 | 0.1714631 |
| **PVC DINP-LDPE** | -130.658 | -440.18561 | 178.869609 | 0.5854132 |
| **Glass-LDPE** | 47.3035 | -262.22411 | 356.831109 | 0.9862833 |
| **PP-HDPE** | 34.1165 | -275.41111 | 343.644109 | 0.9968421 |
| **PVC DEHP-HDPE** | -266.4755 | -576.00311 | 43.052109 | 0.0910795 |
| **PVC DINP-HDPE** | -174.6945 | -484.22211 | 134.833109 | 0.3370214 |
| **Glass-HDPE** | 3.267 | -306.26061 | 312.794609 | 1.0000000 |
| **PVC DEHP-PP** | -300.592 | -610.11961 | 8.935609 | 0.0565026 |
| **PVC DINP-PP** | -208.811 | -518.33861 | 100.716609 | 0.2086179 |
| **Glass-PP** | -30.8495 | -340.37711 | 278.678109 | 0.9980254 |
| **PVC DINP-PVC DEHP** | 91.781 | -217.74661 | 401.308609 | 0.8322265 |
| **Glass-PVC DEHP** | 269.7425 | -39.78511 | 579.270109 | 0.0869531 |
| **Glass-PVC DINP** | 177.9615 | -131.56611 | 487.489109 | 0.3222824 |

## Zusammenfassung

Die Meeresverschmutzung durch Plastik zählt zu den größten Problemen des Anthropozäns. Aufgrund der großen wirtschaftlichen Nachfrage wächst die globale Plastikproduktion jährlich. Der Eintrag von Plastik in die marinen Lebensräume wird inzwischen auf 4.8 - 12.7 Millionen Tonnen pro Jahr geschätzt. Die ins Meer gelangten Plastikteile werden unverzüglich von Mikroorganismen und anderen Biofilm-bildenden Organismen besiedelt. Diese bilden in kürzester Zeit dichte und diverse Biofilmstrukturen auf der Plastikoberfläche. Der Einfluss von Sonnenstrahlung auf Biofilm-bildende Prozesse auf verschiedenen Plastiktypen, ist noch weitgehend unbekannt. In dieser Studie wurden fünf verschiedene Plastikarten, sowie Glas für bis zu 2 Monate im Oberflächenwasser der Nord-Adria, vor der Küste von Rovinj, Kroatien inkubiert, um den Einfluss von Sonnenstrahlung auf die mikrobielle Biofilmbildung zu untersuchen. Hierfür wurden die Plastik- und Glasstücke unter licht- und dunkel- Bedingungen im Oberflächenwasser inkubiert und jeweils nach einer Woche, einem Monat und zwei Monaten beprobt. Die Struktur des entstandenen Biofilms wurde in dieser Zeit durch Raster-Elektronenmikroskopie analysiert. Genetische Unterschiede in der Zusammensetzung des Biofilms wurden durch Sequenzierung des 16S rRNA Genabschnitts festgestellt. Die im Meer inkubierten Plastiktypen waren bereits nach einer Woche mit einem dicken und organismisch-diversen Biofilm überwachsen, welcher je nach Plastiktyp und Lichtbedingung bzw. Inkubationszeitraum unterschiedlich war. Die genetische Analyse des Biofilms zeigte signifikante Unterschiede in der Biofilmzusammensetzung zwischen der licht- und dunklen-Behandlung und den inkubierten Plastiktypen. Es konnte ein klarer Unterschied zwischen den frei lebenden und sessilen Mikroorganismen gezeigt werden. Zwischen den Plastik- und Glasproben konnte keine Differenz in der organismischen Zusammensetzung des Biofilms festgestellt werden. Die beiden Bedingungen (Licht und Dunkel) zeigten klare Abweichungen in der Zusammensetzung und Artenhäufigkeit des Biofilms. Unter Lichtbedingungen dominierten phototrophe Taxa die mikrobielle Biofilm-Gemeinschaft, während unter dunklen Bedingungen lichtsensitive Taxa häufiger vorkamen. Die Diversitätsindices (Shannon Index/Artenvielfalt) zeigten zu Beginn des Experiments und nach zwei Monaten Inkubationszeit eine signifikant höhere Diversität/Artenvielfalt unter dunklen Bedingungen, mit der Ausnahme der Artenvielfalt zu Beginn des Experiments, die keine signifikanten Unterschiede zwischen den Licht-Bedingungen zeigte. Außerdem wurde statistisch ein

signifikanter Unterschied zwischen den Artenzusammensetzungen der Licht und Dunkel Bedingungen festgestellt. Die beiden PVC Proben unterschieden sich in ihrer Artenzusammensetzung deutlich von allen anderen Plastiktypen. PVC DEHP zeigte außerdem nach einer Woche Inkubationszeit eine signifikant niedrigere Artenvielfalt im vergleich zu den anderen Substraten, was impliziert, dass die Oberflächenstruktur oder die beigemischten Weichmacher die Bildung eines Biofilms beeinflussen könnte. Verschiedene bekannte Kohlenwasserstoff- bzw. Öl-abbauende Mikroorganismen wurden im Biofilm der Plastikproben gefunden, jedoch nicht im umliegenden Oberflächenwasser. Dies gibt Grund zur Annahme das Plastik eventuell von manchen Mikroorganismen als Kohlenstoff-quelle gesehen wird und sich diese deswegen am Plastik ansiedeln. Nichtsdestotrotz konnten während des Experiments weder Plastik-abbauende Prozesse noch Indikatoren für diese beobachtet werden.