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Microbial colonization and degradation of polyethylene and biodegradable plastic bags in temperate fine-grained organic-rich marine sediments

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A B S T R A C T

To date, the longevity of plastic litter at the sea floor is poorly constrained. The present study compares colonization and biodegradation of plastic bags by aerobic and anaerobic benthic microbes in temperate fine-grained organic-rich marine sediments. Samples of polyethylene and biodegradable plastic carrier bags were incubated in natural oxic and anoxic sediments from Eckernförde Bay (Western Baltic Sea) for 98 days. Analyses included (1) microbial colonization rates on the bags, (2) examination of the surface structure, wettability, and chemistry, and (3) mass loss of the samples during incubation. On average, biodegradable plastic bags were colonized five times higher by aerobic and eight times higher by anaerobic microbes than polyethylene bags. Both types of bags showed no sign of biodegradation during this study. Therefore, marine sediment in temperate coastal zones may represent a long-term sink for plastic litter and also supposedly compostable material.

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1. Introduction

The production of plastic material has constantly increased over the past 50 years (PlasticsEurope, 2013) and contributes to the resulting pollution of marine environments (Derraik, 2002). Polyethylene (PE) is a major component of plastic waste found in oceans (Morét-Ferguson et al., 2010; Zettler et al., 2013), on shorelines, at the ocean surface, and on the sea floor, mostly in the form of carrier bags (Barnes et al., 2009).

Marine plastic pollution may harm marine organisms via ingestion or entanglement, and may favor the dispersal of invasive species (Gregory, 2009). Additionally, the release of hazardous chemicals, including additives (components of plastic) and the accumulation of hydrophobic toxins (adsorbed onto plastic from surrounding sea water) were reported (Teuten et al., 2009). Due to the longevity of plastic in the environment and the ensuing long-term threat to organisms, alternatives to these synthetic polymers were developed and tested (O'Brine and Thompson, 2010). Different types of degradable plastics are commercially available, such as natural plastics produced by microorganisms, or plastics with polymer blends, such as starch and photo-biodegradable plastics (Shah et al., 2008). To date, studies focusing on different types of degradable plastics, marine environments and locations have been conducted by Accinelli et al. (2012); Andrady et al. (1993), Rutkowska et al. (2002) and Tosin et al. (2012). The mentioned studies obtained conflicting results and it remains unclear whether degradable plastics are less harmful.

Microbes are ubiquitously abundant in the marine environment, capable of decomposing complex organic matter. Hence, the question arises whether microbial degradation of plastic litter is possible and whether it has the capacity to counteract the gradual accumulation of plastics in marine environments. So far, most studies on the microbiological colonization and degradation of plastic are restricted to the upper ocean layer. Zettler et al. (2013) described a diverse microbial community growing on plastic material from North Atlantic surface water, which differed from the bacterial composition of the surrounding water. Pits in the plastic surface of the same size and shape as that of bacteria were interpreted as possible features of biodegradation. In another study, polyethylene (PE) incubated for 20 months in 2 m water depth in the Baltic Sea showed no biodegradation (Rutkowska et al., 2002).

The initial positive buoyancy and the hydrophobicity of PE may be altered by UV–radiation, oxidation, high temperatures (Andrady, 2011, Shah et al., 2008), and biofilm formation (Muthukumar et al., 2011). After approximately three weeks of floating at the ocean surface,

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PE bags start to sink below the seawater–air interface (Lobelle and Cunliffe, 2011). Adhesion of more particles onto the PE surfaces (Gregory, 2009) and wind-induced downwelling cause bags to sink further, until eventually they settle onto the seafloor (Kukulka et al., 2012). As light decreases with depth, it is likely that the rate of abiotic plastic degradation decreases in deep waters (Barnes et al., 2009). Although little is known about the dimension of plastic pollution of the seabed at depths >30 m (Watters et al., 2010), plastic debris have been found on the seafloor of every ocean (Barnes et al., 2009). Galgani et al. (1996) found up to 78 litter items/ha on the continental slope and bathyal plain of the northwestern Mediterranean Sea. Here, more than 70% of the total debris consisted of plastic bags. Once on the ocean floor, plastic material is buried into the seabed by ongoing sedimentation and/or bioturbation. During burial it passes the thin oxic sediment surface layer before reaching the anoxic sediment below. It is unknown how degradation rates of plastic in sediments are affected by the lack of oxygen and light. As microorganisms in the sediment largely control carbon sequestration and nitrogen conversion (Wu et al., 2008) and therefore play an important role in marine biogeochemical cycles (Strom, 2008), it is crucial to investigate their possible contribution to the biological degradation of deposited plastic.

To our knowledge, the only data available on benthic microbial settlement and degradation of plastic debris were published by Kumar et al. (2007) and Tosin et al. (2012). Kumar et al. (2007) investigated plastic degradation in mangrove soil. Tosin et al. (2012) tested plastic degradation in a simulation of the eulittoral and sublittoral zone. However, tropical mangrove soils and coarse-grained sandy sediments represent the ocean floor only to a limited extent (Fütterer, 2000). So far the microbial colonization and degradation of plastic in temperate, fine-grained sediments, which are more common at continental shelves, is unknown. However, this information is essential to estimate the fate of buried plastic in wide regions of the seafloor.

The present study focuses on the fate of PE and compostable carrier bags in oxic and anoxic coastal fine-grained, organic-rich sediment. A laboratory experiment was conducted to examine the colonization intensity of aerobic and anaerobic microorganism on the two plastic bags, the microbial alteration of the bag surfaces, and their mass changes.

2. Material and methods

2.1. Preparations of carrier bag materials

Commercially available polyethylene (PE) carrier bags and biodegradable bags produced by Melitta Europa GmbH & Co. KG were used. The putatively biodegradable bags ("COMP" hereafter) consist of >50% biodegradable polyester, >20% corn starch and additional, undisclosed components (personal correspondence with manufacturer). These bags meet the standards for compostability EN 13432 according to DIN CERTCO. From both bag types, 1 × 2 cm-samples were prepared and fixed to a short stainless steel wire to provide negative buoyancy. The plastic samples were sterilized with 70% ethanol for 2 min and washed with ultra-purified water for 2 min before the experiment was started.

2.2. Study site and sediment sampling

Sediment cores were obtained in February 2013 with a miniaturized *mul*ti *c*orer (MUC) onboard the research vessel "Littorina" at the long-term monitoring station "Boknis Eck" (54°31.2′ N, 10°02.5′ E, 28 m water depth) located in the Eckernförde Bay, Baltic Sea (Germany). These sediments are rich in organic carbon (5–6 wt.%) with an annual sedimentation rate of 1.4 mm yr^{-1} (Whiticar, 2002). Oxygen penetration depth into the sediment is around 0.1–0.2 cm (Preisler et al., 2007). Microbial cell numbers in the surface sediment layer (0–1 cm) range between 1.7 and 7.2 × 10^9 cells/g dry weight with the highest numbers found during winter and early spring compared to cell numbers in fall (Meyer-Reil, 1983). Deeper sediment layers (~25 cm) feature cell numbers of ca. 1.3 × 10^8 cells cm^{-3} (Treude et al., 2005). More details of Eckernförde Bay are summarized in Table 1.

2.3. Sediment slurry preparation

After MUC sampling, sediment cores were sub-sampled immediately on board for the preparation of oxic and anoxic sediment–seawater mixtures (slurries).

2.3.1. Oxic sediment slurry.

For the oxic slurries, the light colored top (0–1 cm sediment depth) of the MUC cores was transferred into autoclaved one liter Duran® glass bottles, which were closed with autoclaved cotton plugs and cooled at 5 °C until slurry preparation was conducted (one month later). Sea salt medium was chosen in order to prepare the slurries. Salinity and pH of the medium were adjusted to the Kiel Bight values of around 18 and 8.3, respectively. Afterwards the medium was autoclaved. Sediment and sea salt medium were mixed in a 1:1 ratio (vol/vol).

2.3.2. Anoxic sediment slurry.

For the anoxic slurries, the black, reduced (sulfidic smell) sediment from 5 to 10 cm sediment depth of the MUC samples was transferred headspace-free into autoclaved one liter Duran® glass bottles. The bottles were closed with autoclaved butyl stoppers and screw caps, and were kept at 5 °C until slurry preparation (after one month). In order to prepare the anoxic sediment slurries, the DSMZ (German Collection of Microorganisms and Cell Cultures) 196 modified *Desulfobacter postgatei* medium was used. Magnesium and calcium concentrations were adjusted to match seawater ($MgCl_2(6H_2O)$ 10.83 g l^{-1}; $CaCl_2(2H_2O)$ 1.53 g l^{-1}). No additional carbon source was added. Salinity was adjusted to Baltic Sea value. In order to maintain anoxic conditions in both medium and sediment during slurry mixing, the slurry preparation was conducted inside a glovebox (N_2 gas) with an oxygen level <1 ppm. For slurry preparation, sediment and medium were combined in a 1:1 ratio (vol/vol). All sediment slurries were stored at 5 °C prior to the experiment start.

Table 1
Characteristics of Eckernförde Bay.

Parameter	Data
Water depth	28 m
Carbon content in sediment	5–6 wt.% (Whiticar, 2002)
Sedimentation rate	1.4 mm yr^{-1} (Whiticar, 2002)
Porosity	0.88 (Preisler et al., 2007) Up to 0.92 (Treude et al., 2005)
O_2 penetration depth	0.1–0.2 cm (Preisler et al., 2007)
Temperature max. at 25 m	11–13 °C October (Lennartz et al., 2014) 1–2 °C March (Lennartz et al., 2014)
Oxygen concentration max. at 25 m	290–350 μmol l^{-1} February, March (Lennartz et al., 2014)
Oxygen concentration min. at 25 m	0–20 μmol l^{-1} September, October (Lennartz et al., 2014)
Microbial cell number in sediment	1.7 × 10^9 and 7.2 × 10^9 cells per g of dry weight, 0–1 cm sediment depth (Meyer-Reil, 1983) 1.2 × 10^8 to 1.4 × 10^8 cells cm^{-3}, 24–26 cm sediment depth (Treude et al., 2005)
Sulfate reduction rate	4–5.4 mmol SO_4^{2-} m^{-2} d^{-1} (integrated over 0–25 cm sediment depth) (Treude et al., 2005) 32.4 nmol SO_4^{2-} cm^{-2} d^{-1} (integrated 0–18 cm sediment depth) (Bertics et al., 2013)

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