Pantos[1], O., Audrezet[2], F., Doake[1], F., Donaldson[3], L., Dupont[1], P., Gaw[4], S., Kingsbury[1], J.K., Weaver[1], L., Lear[5], G., Maday[5], S., Northcott[6], G., Pochon[2,5], X., Smith[3], D.A., Theobald[3], B., Wallbank[5], J., Zaiko[2,5], A.

E-mail: olga.pantos@esr.cri.nz

Institute of Environmental Science and Research (ESR),

ESR, Christchurch Science Centre, 27 Creyke Road, Ilam, Christchurch 8041, New Zealand

[1]Institute of Environmental and Science Research, 27 Creyke Road, Ilam, 8041, NZ. [2]Cawthron Institute, 98 Halifax Street, Nelson, NZ. [3]Scion, 49 Sala Street, Rotorua 3010, NZ. [4]School of Physical and Chemical Sciences, University of Canterbury, Christchurch, NZ. [5]School of Biological Sciences, University of Auckland, 3a Symonds Street, Auckland 1010, NZ. [6]Northcott Research Consultants Limited, Hamilton NZ.

A pilot study to determine the potential impacts of plastics on Aotearoa-New Zealand's marine environment

Introduction

Plastic of all sizes, from nano- to macro-, and of all different polymer types are found in our oceans around the world. They present a range of threats to the environment at all sizes, and may enter the food web at different trophic levels. Impacts from ingestion range from causing physical internal damage to causing a false sense of satiation, resulting in subsequent implications for animal health and fitness. Inherent or acquired chemicals associated with the plastics may also pose a significant risk to the biota at different trophic levels both following direct consumption of the plastics and from bioaccumulation and biomagnification through the food chain. They provide a new substrate for the development of biofilms, and their buoyant and resilient nature means they pose a threat to ecosystems through their role in the translocation of invasive species and pathogens in the oceans.

Figure 1. Plastics enter our oceans through both land-based and sea-based activities. The weathering of plastic through biological, physical and mechanical processes cause them to fragment into increasingly smaller pieces; microplastics and nanoplastics.

This is the first study to examine the effect of polymer type on the biofilm communities that form on marine plastics in Aotearoa-New Zealand (A-NZ) waters, improving our understanding of their potential impacts. Here we present the results of a 3-month exposure experiment, where the bacterial and fungal communities of 2 polymers were examined.

Objectives

- Identify whether polymer type influences the microbial communities and higher order species that form the plastisphere.
- Determine changes to plastics during exposure to the marine environment which may influence their fate.

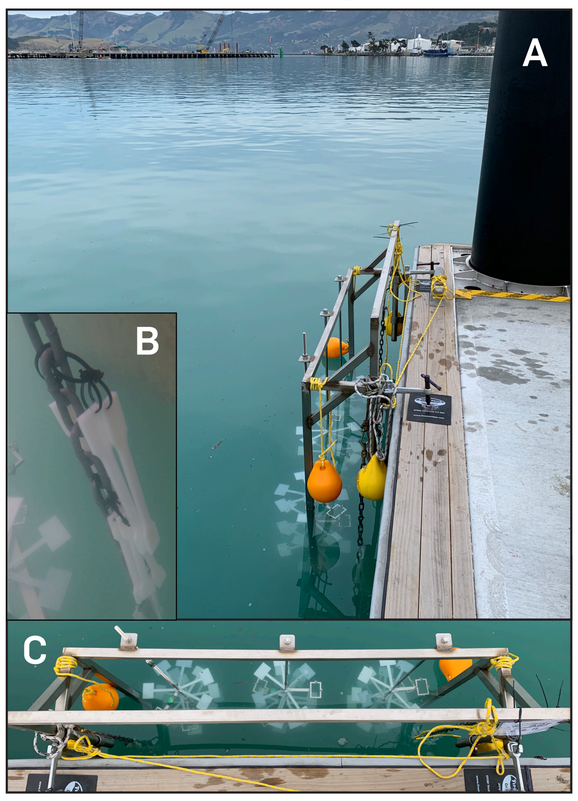
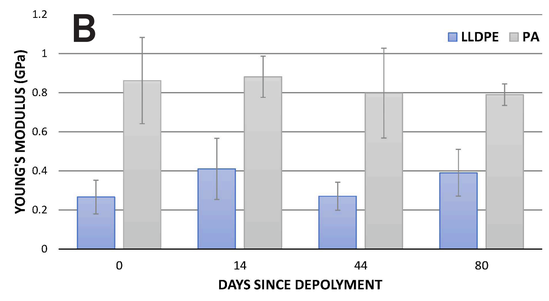


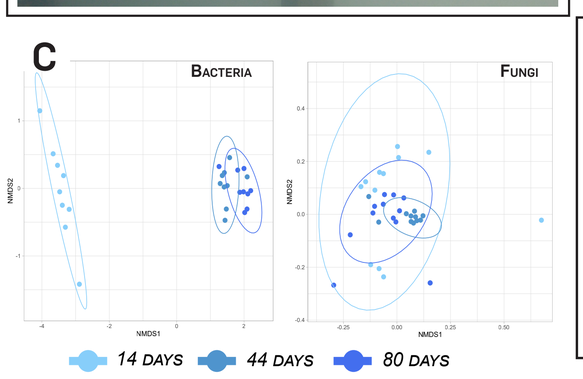
Figure 2. Experimental deployment site within Lyttelton Port of Christchurch, NZ (Lat;Long: 43.6075815; 172.723340). A) Steel structure attached to the pontoon holds vertical rods with experimental plastics in place, allowing movement up and down with the tide and continuous submersion. B) Changes in the tensile properties of the polymers over time were determined using standard shapes. C) Three vertical rods with experimental plastics and glass controls attached.

Materials & Methods

- A system made of marine grade stainless steel (316) was designed to allow the submersion of plastics in surface waters. Bespoke injection moulded plastics (nylon 6 (PA) and linear low-density polyethelyne (LLDPE)) with known additives were affixed to the structure which was attached to the side of a pontoon (Fig. 2) at the Lyttelton Port of Christchurch, Canterbury, A-NZ. Glass supported by stainless steel (316) frames were used as an inert control substrate. Triplicates of each substrate type were distributed evenly over three rods, and three depths (20, 40, 60 cm). Temperature/light loggers placed at each height.
- Mechanical properties (modulus, max stress and elongation at break) of the plastics were carried out (ISO 527-2).
- Total genomic DNA extraction (Qiagen DNeasy PowerSoil) ⇨ 16S rRNA gene V3-V4 (341F/805R) and ITS2 rRNA gene (fITS7/ITS4) amplified ⇨ sequenced (MiSeq 2x300 paired end reads).
- Sequence processing: conducted with Dada2 using the Silva v128 database.
- Sequence analysis: conducted with phyloseq and vegan in R v3.5.1.

Results:





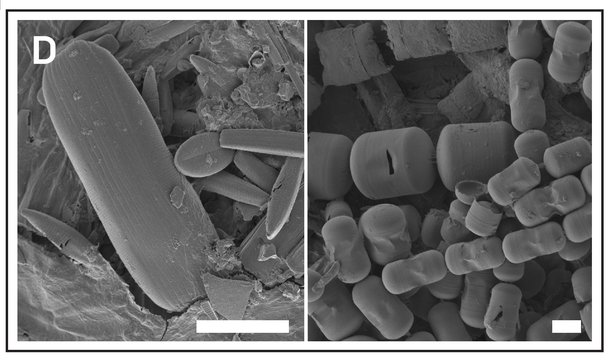


Figure 3. A) Macroscale differences are evident between the upper and lower surface biofilms of plastic and glass at 3 months. B) Mechanical testing found no significant change (n = 5) in the stiffness (modulus) of the plastics over 3 months. C) Biofilm bacterial communities showed change over time; fungal communities did not. Ellipses = 95% CI. D) Diatoms identified by SEM and 18S rRNA sequence analysis identified 7 genera (data not shown) predominated the biofilm matrices (Scale bars = 20 µm).

- Biofilms rapidly developed on all substrate types (Fig. 3A).
- No light or temperature difference occurred between the 3 depths, and no depth effect on microbial community composition was observed.
- Mechanical properties (Fig.3B) of both plastics did not change significantly over time.

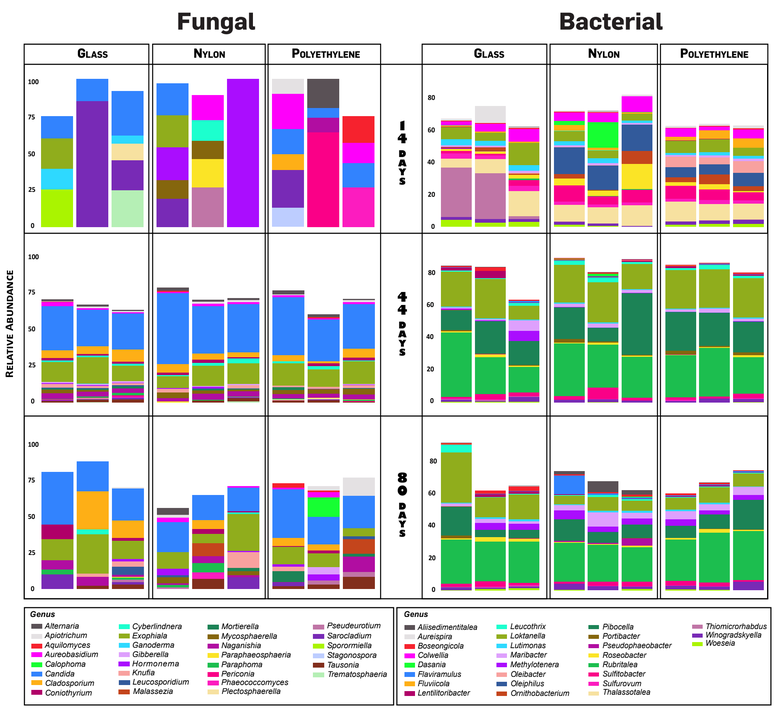


Figure 4. Relative abundance of fungal and bacterial genera (top 4 and 5 genera, respectively) present within the biofilm communities at the 3 sampling times.

- Biofilm microbial communities were independent of those in the water column.
- Bacterial but NOT fungal biofilm communities changed over time (Fig. 3C).
- Material-specificity of bacterial communities at 14 days only.
- Alpha-diversity of both fungal and bacterial communities did not change with age or material.

Discussion/Conclusions

- Although the mechanical properties of PA and LLDPE did not change significantly over 80 days exposure in seawater, microscale changes may occur which may influence their interaction with microbial biofilms. Further SEM analysis is being carried out.
- The substrate-type effect seen in bacterial communities at 14 days may be a result of differences in surface texture and colour of the polymers and glass. Disappearance by 44 days, could be due to the dense biofilm present, screening the influence of the substrate.
- The nonspecific association of fungi suggests the absence of selective pressure from either the substrate or young biofilm. Loss of conformity at 80 days is unclear.
- The absences of an anticipated polymer-type effect due to the hydrophobic/hydrophilic nature of LLDPE/PA, but may have occurred earlier in the biofilm community succession.

Acknowledgements: We are grateful to Lyttelton Port Company for granting continued support, access and permission to use their pontoon for deployment. We thank Evelyn Hollaus and Erin McGill (ESR) for technical assistance. Funding was provided by Ministry of Business, Innovation and Employment (MBIE) Endeavour Fund C03X1802.