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Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula

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Abstract

Marine plastic pollution has a range of negative impacts for biota and the colonization of plastics in the marine environment by microorganisms may have significant ecological impacts. However, data on epiplastic organisms, particularly fungi, is still lacking for many ocean regions. To evaluate plastic associated fungi and their geographic distribution, we characterised plastics sampled from surface waters of the western South Atlantic (WSA) and Antarctic Peninsula (AP), using DNA metabarcoding of three molecular markers (ITS2, 18S rRNA V4 and V9 regions). Numerous taxa from eight fungal phyla and a total of 64 orders were detected, including groups that had not yet been described associated with plastics. There was a varied phylogenetic assemblage of predominantly known saprotrophic taxa within the Ascomycota and Basidiomycota. We found a range of marine cosmopolitan genera present on plastics in both locations, i.e., *Aspergillus*, *Cladosporium*, *Wallemia* and a number of taxa unique to each region, as well as a high variation of taxa such as Chytridiomycota and Aphelidomycota between locations. Within these basal fungal groups we identified a number of phylogenetically novel taxa. This is the first description of fungi from the Plastisphere within the Southern Hemisphere, and highlights the need to further investigate the potential impacts of plastic associated fungi on other organisms and marine ecosystems.

KEYWORDS
antarctica, DNA metabarcoding, fungi, marine, plastics, plastisphere

1 | INTRODUCTION

The oceans contain an estimated 15–51 trillion pieces of floating plastics (van Sebille et al., 2015) which have a significant impact on marine wildlife through ingestion, entanglement and asphyxiation (Gall & Thompson, 2015; Gregory, 2009; Li, Tse, & Fok, 2016). Within aquatic systems, plastic pollution forms floating artificial ecosystems, referred to as the "Plastisphere" (Amaral-Zettler, Zettler, & Mincer, 2020; Zettler, Mincer, & Amaral-Zettler, 2013). The hydrophobic nature of plastics stimulates biofilm formation and allows the establishment of a succession of prokaryotic and eukaryotic micro and macro organisms (Carpenter & Smith, 1972; Oberbeckmann, Loeder, Gerdts, & Osborn, 2014; Reisser et al., 2014). The impacts of these biofilms may include spread of pathogenic organisms

or invasive species (Barnes, 2002; Reisser et al., 2014; Zettler et al., 2013) and the increase in palatability of plastics for ingestion (Coppock et al., 2019; Hodgson, Bréchon, & Thompson, 2018; Procter, Hopkins, Fileman, & Lindeque, 2019).

Molecular surveys have revolutionised our understanding of fungi in the marine environment (Amend et al., 2019; Gladfelter, James, & Amend, 2019; Grossart et al., 2019; Richards, Jones, Leonard, & Bass, 2012; Richards et al., 2015) but we know little about fungal diversity within epiplastic communities. In marine environments, fungi occupy a complex range of ecological niches but in plastic biofilms little is known about their functional role. Several fungal taxa from marine and terrestrial environments are able to degrade plastic polymers (Paço et al., 2017; Russell et al., 2011) and we know that although community composition is highly variable, fungi are early colonisers of plastics, meaning they are potentially important in biofilm formation (De Tender et al., 2017). Moreover, marine plastics are often covered in polysaccharide-rich diatom biofilms (Lacerda et al., 2019), this could explain the attachment and association of fungi with biofilms on plastics (Kettner, Rojas-Jimenez, Oberbeckmann, Labrenz, & Grossart, 2017; Oberbeckmann, Osborn, & Duhaime, 2016). Recent studies have directly linked fungi to the degradation of organic matter (Cunliffe, Hollingsworth, Bain, Sharma, & Taylor, 2017;Ortega-Arbulú, Pichler, Vuillemin, & Orsi, 2019), and in the open ocean fungi may attach to marine snow particles (Bochdansky, Clouse, & Herndl, 2017; Duret, Lampitt, & Lam, 2020). Other marine debris such as wood also harbour fungi, suggesting that Fungi are well adapted to live on different types of floating marine substrates (Kettner, Oberbeckmann, Labrenz, & Grossart, 2019; Kettner et al., 2017). The abundance and diversity of fungi in coastal waters varies seasonally in response to environmental variables such as particulate organic carbon (Duan et al., 2018; Taylor & Cunliffe, 2016), which could also affect how and which fungal taxa colonize plastics.

Assessing biodiversity of the Plastisphere is fundamental to increase our knowledge of plastic associated organisms, their biogeography and potential impacts (Amaral-Zettler et al., 2015; Oberbeckmann, Löder, & Labrenz, 2015; Zettler et al., 2013). We know little about plastics and fungi in the oceanic waters of the South Atlantic and Southern Oceans. These two regions are linked by the Antarctica Circumpolar Current (ACC), which branches towards the equator (Falkland/Malvinas Current) and enters the South Atlantic Ocean reaching the Brazilian coast (Matano, Palma, & Piola, 2010), and also by near-surface currents that flow in the opposite direction (Wichmann, Delandmeter, & Sebille, 2019). Considering plastics could be transported by oceanic currents (Eriksen et al., 2014), the associated epiplastic organisms may also be dispersed between areas. Antarctica is often viewed as a pristine environment yet our recent study identified a range of plastic types (Lacerda et al., 2019).

Within Antarctic coastal waters, a diversity of fungi species have been recorded (Bridge & Spooner, 2012; Rosa, 2019), with several endemic taxa being found associated with macroalgae (Loque et al., 2010). In the western South Atlantic (WSA), only a few studies have described the fungal diversity in marine ecosystems, with more

focus on using fungi as indicators of pollution in the marine environment (Azedo Loureiro, De Queiroz Cavalcanti, Neves, & De Oliveira Passavante, 2005; Hagler & Mendonça-Hagler, 1981). Molecular techniques can reveal a much higher diversity of fungi, particularly parasitic and pathogenic taxa which are difficult to isolate without host taxa (Richards et al., 2012). Several studies looking at general eukaryotic diversity on plastics have used a general 18S ribosomal RNA (rRNA) marker to profile communities with high-throughput sequencing, identifying a variety of fungal taxa within their data sets (Debroas, Mone, & Ter Halle, 2017; Kettner et al., 2019; Kirstein, Wichels, Krohne, & Gerdts, 2018; Oberbeckmann et al., 2016). However, the 18S rRNA gene has limited taxonomic resolution for some fungal groups (Richards et al., 2012; Stoeck et al., 2010;Taylor & Cunliffe, 2014) and may give different profiles depending on sequenced region (De Filippis, Laiola, Blaiotta, & Ercolini, 2017). Using fungi-specific primers, such as those targeting the Internal Transcribed Spacer regions (ITS) of the rRNA operon, ensures a high proportion of sequence reads from fungi and provide good resolution for higher fungi within Ascomycota and Basidiomycota (Andreakis et al., 2015; Rämä et al., 2016).

Our study is the first description of the taxonomic composition and functional guild classification of fungi from the Plastisphere of oceanic surface waters within the Southern Hemisphere. We aimed to evaluate the diversity of fungi associated plastics and their geographical distribution between two Southern Hemisphere locations, the WSA and the AP. To profile the total fungal community, a multimarker metabarcoding approach was used on marine micro and meso plastics sampled from subtropical waters of WSA and polar waters around the AP. We extracted DNA from the plastic and amplified the 18S rRNA gene V4 and V9 hypervariable regions using general eukaryotic PCR primers and ITS2 region using fungal specific primers. We hypothesised that (a) multiple taxa of fungi would be associated with plastics in the WSA and AP, as had been suggested in other areas such as the North Sea; and (b) there would be geographical difference in fungal community composition associated with plastics between WSA and the AP region, with taxa unique to each region, as noted for algal and bacterial communities in the Plastisphere of the Northern Hemisphere.

2 | MATERIALS AND METHODS

2.1 | Plastic sampling and characterization

Plastic samples were collected from surface waters of two oceanic regions, the subtropical western South Atlantic (WSA) and around the Antarctic Peninsula (AP), as part of the TALUDE and INTERBIOTA Projects. At each sampling point, manta trawls (330 μm mesh) were performed for 15–55 min at the air-ocean interface, at a speed of 3 knots. Samples were collected at 10 stations at WSA and 12 stations around the AP (Figure 1). Within the WSA, we sampled from stations close to the Brazilian coast and stations near the shelf break in a latitudinal gradient from 26°S to 34°S. In Antarctica, sampling stations were situated to the west of the AP from 61°S to 64°S. After sampling,

FIGURE 1 Sampling stations for marine plastics from surface waters in the western South Atlantic (WSA) and Antarctic Peninsula (AP)
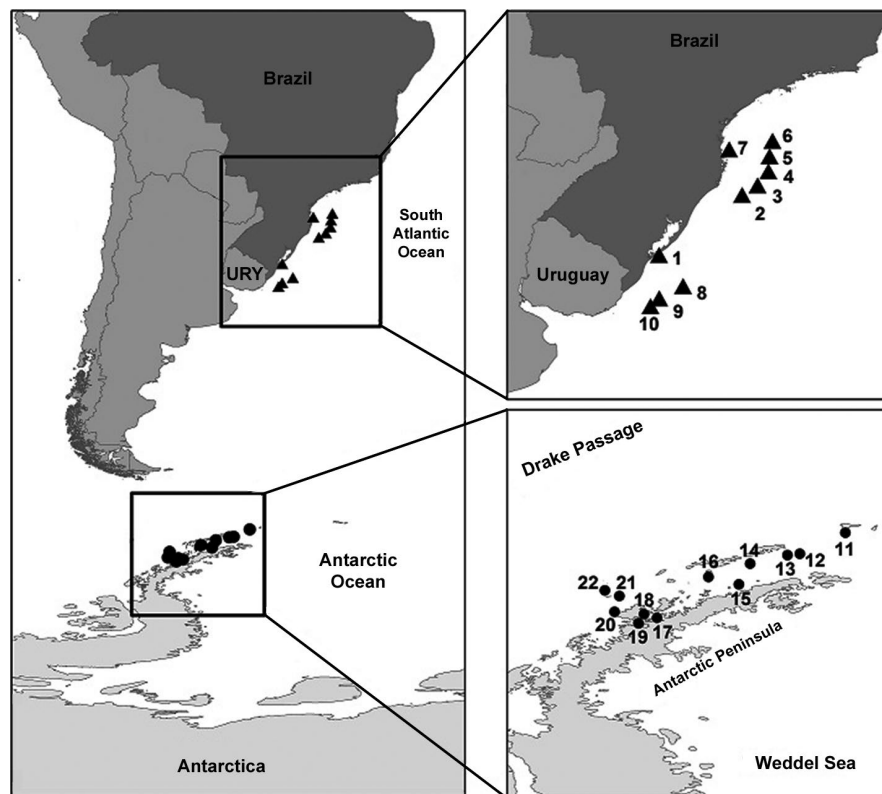
the collected material was placed in aluminium bags and immediately frozen at −20°C. In the laboratory, each sample was separately thawed and placed in a sterile container filled with artificial sterile salt water (salinity 35, temperature ~ 4°C) for manual separation of floating plastic pieces and biomass (Reisser et al., 2013). Each sample was visually examined and when found, plastics were picked up using sterile forceps, posterior characterization of their size (measured over their largest cross-section) was measured with calipers. Based on published definitions, plastics < 5 mm were classified as microplastic and plastics between 5–200 mm were classified as mesoplastic (GESAMP, 2015). In addition, shape was also recorded (line, fragment, sphere). Each plastic fragment was given an individual identifier number. Each piece of plastic was placed individually in a microcentrifuge tube with ethanol P.A. (MERK) and frozen at −20°C to preserve biofilm DNA, large soft pieces of plastic or lines were folded to fit in the tubes.

Using the identifier number and a random number generator, 58 plastic fragments were randomly selected for DNA analysis. After DNA extraction from those that were not destroyed during the process, 28 were selected for polymer composition following the methods of Lacerda et al. (2019). In brief, the polymer composition was determined through Fourier Transform Infrared Spectroscopy (FTIR) with a SHIMADZU, model Prestige 21, using a diffuse reflectance module, 24 scans and 4 cm^{-2} resolution.

2.2 | DNA extraction

Plastics were rinsed in sterile artificial seawater to remove loosely associated organisms before DNA extraction. The total DNA of the biofilm formed on the marine plastics was extracted using PowerSoil DNA extraction Kits (Qiagen). This kit has previously been shown to be suitable for recovering microbial DNA from the surface of plastics in a DNA methods comparison study from micro- and mesoplastics (Debeljak et al., 2017). In this study the kit used here gave similar, and in some cases better DNA yield, to phenol-chloroform extraction and provided the best quality DNA. The kit has also been used widely in DNA extraction from Fungi and performs well on analysis of mock communities (Hermans, Buckley, & Lear, 2018). Plastic fragments were transferred to extraction tubes and the extractions were carried out according to the manufacturer's instructions, with the exception of the first step (digestion), where we added 10 μl (1,000 U/μl) of lysozyme to improve extraction efficiency (Debeljak et al., 2017), and the last step where DNA was eluted in a lower buffer volume (30 μl). The quality and concentration of the extracted DNA were checked by spectrophotometry using a Biodrop DUO (Harvard Bioscience).

2.3 | Polymerase chain-reaction (PCR) and high-throughput sequencing

To amplify the V9 region of the 18S rRNA gene (Amaral-Zettler, McCliment, Ducklow, & Huse, 2009) the primers 1391F (5′-GTACACACCGCCCGTC-3′) and Euk B (5′-TGATCCTTCTGCAGGTTCACCTAC-3′) were used, and PCR was carried out under the following conditions: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 55°C 45 s and 72°C 90 s, with a final 5 min extension at 72°C; to amplify the V4 region of the 18S

rRNA gene (Stoeck et al., 2010) the primers were TAReuk454FWD1 (5′-CAGCASCYGCGGTAATTCC-3′) and TAReukREV3 (5′-ACTTTCGTTCTTGATYRA-3′), and PCR conditions were: 95°C for 7 min, followed by 40 cycles of 95°C for 30 s, 48°C 45 s and 72°C 90 s, with a final 7 min extension at 72°C. Finally, to amplify the ITS2 region (Ihrmark et al., 2012), a semi-nested PCR approach was used. The first PCR was carried out using the fungal specific forward primer ITS1f (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and general primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). PCR products from the first PCR were diluted 1:50 and 1μl used in the second PCR with the primer gITS7 (5′-GTGARTCATCGARTCTTTG-3′) and ITS4; both first and semi-nested PCR were run with the same conditions of 94°C during 5 min, followed by 30 cycles of 94°C for 30 s, 53°C 45 s and 72°C 90 s, with a final 7 min extension at 72°C. All PCRs were carried out in 25 μl reactions using GoTaq Flexi G2 polymerase containing 5 μl of Gotaq FlexiBuffer, 2 μl of $MgCl_2$ (2 mM), 0.5 μl of DNTPs (10 mM, final concentration) (Thermo-scientific), 0.5 μl of primers (forward and reverse, 0.2 mM each), 0.125 of Taq DNA polymerase enzyme, 1 μl of DNA template and 15 μl of ultrapure water. Successful amplification was confirmed with gel electrophoresis. Negative controls containing 1 μl ultrapure water instead of template were run with all PCR steps and were taken through to sequencing. All PCR primers contained adapters for the Illumina Nextera XT workflow as well as 12 random bases on the forward primer to increase sequence cluster diversity on the Illumina slide (see Table S1 for full primer constructs). Amplifications were purified using 0.8x Ampure XP beads as per the manufacturer's instructions (Beckman Coulter). Products were then quantified using a Promega high sensitivity Quanti-Fluor kit (Promega), and diluted to 10 ng/μl. 18S V9, V4 and ITS2 amplicons were then pooled in equimolar concentrations and indexing was performed using an Illumina Nextera barcoding kit as per the manufacturer's instructions. Indexed libraries were then pooled, quantified and diluted to 4 p.m. and run on Illumina Miseq using the V3 2 × 300 bp Illumina sequencing kit (Illumina) at the University of York Genomics and Bioinformatics laboratory.

2.4 | High-throughput sequence analysis

Sequences were analysed using a combination of USEARCH v8 (32Bit) (Edgar, 2010) and QIIME v 1.8.0 (Caporaso et al., 2010). Forward and reverse reads were merged using USEARCH. Cutadapt (Martin, 2011) was used to separate each individual primer set from the data set, removing primers and adapters. For each primer set fastq files were quality filtered (low quality, expected error > 0.5 and short sequences < 200 bp), with a 200 bp minimum length for ITS2 and length truncated for 18S (370 bp for V4 and 150 bp for V9) and then converted to FASTA files. The FASTA files were dereplicated, abundance sorted and had their singleton sequences removed. Operational taxonomic units (OTUs) were clustered using the UPARSE clustering algorithm at 97% (Edgar, 2013). Chimeras were filtered using UCHIME (Edgar, Haas, Clemente, Quince, & Knight, 2011), and OTUs

were then mapped back to the original reads and an OTU table produced. For ITS2 sequences, taxonomy was assigned to OTUS using BLAST against the UNITE ITS 99% clustered sequence database (7.1 2016–11–20 release) (Kõljalg et al., 2005) and a custom ITS database containing all ITS sequences across all taxonomic groups downloaded from Genbank which was used to identify nonfungal OTUs. The custom ITS database was made from ITS sequences downloaded from the National Center for Biotechnology Information database (NCBI – accessed 17/05/2018). Sequences were filtered to contain full length ITS and partial ITS2 fragments > 100 bp and no more than two consecutive N bases. The database was then dereplicated of identical sequences and clustered at 99% before both FASTA files and taxonomy were manually formatted for input into QIIME. The 18S V4 and V9 sequences were classified against the 99% clustered SILVA 132 database (Quast et al., 2013) using UCLUST (Edgar, 2010). Sequence data is available in the European Nucleotide Archive under the project accession code PRJEB35146.

2.5 | Taxonomy, alpha and beta diversity analysis

Fungal OTUs were separated from all data sets and detailed taxonomy checked and confirmed by BLAST against the full NCBI database. OTUs appearing in only one sample and with less than two reads were excluded from downstream analyses. Potential trophic guilds were assigned to fungal OTUs using FUNGuild considering only probable and highly probable matches for each taxa (Nguyen et al., 2016). Frequency graphs were constructed based on the frequency of occurrence (number of plastic pieces on which fungal order were present) in each location, separated by marker. To visualise shared and unique orders of fungi between locations, Venn diagrams were constructed using Venny (http://bioinfogp.cnb.csic.es/tools/venny/).

To assess OTU richness of fungi associated with individual fragments of plastic at each location, OTU tables were rarefied to 1,000 reads for ITS2, 300 reads for 18S rRNA V4 and 500 reads for 18S rRNA V9. Statistical analysis was performed in R studio 1.1.456 (R Development Core Team) using the vegan (Oksanen et al., 2019) package. A Kruskal-Wallis test was performed to check for differences between OTU richness per sample between variables (location, size class, shape, and polymer composition) for each marker gene. Dissimilarity matrices between the samples were constructed using a Binary Jaccard index, statistical differences between locations and metadata groups were tested with PERMANOVA with 999 permutations. The Jaccard dissimilarity matrix was used to produce nonmetric multidimensional scaling (NMDS) plots.

2.6 | Phylogenetic analysis of 18S V4 OTUs

From the 18S v4 OTUs from both WSA and AP samples (total 56 fungal sequences) the data was separated into only Dikarya (Ascomycota & Basidiomycota), 22 sequences, and the other data set comprised of Chytrids and other lineages (Chytridomycota,

Mucoromycota, Zoopagomycota and assignments to the Rozellomycota, Aphelidomycota and Cryptomycota), 34 sequences. OTUs were compared to reference sequences from genbank and the nearest uncultured or cultured isolate sequences downloaded. Environmental sequence clusters were also taken from Richards et al. (2015) for comparison, particularly novel lineages and clades. Both data sets were imported into Mega X (Kumar, Stecher, Li, Knyaz, & Tamura, 2018). Alignment was performed in Mega X using MUSCLE (Edgar, 2004). Phylogenetic analysis was performed in Mega X using Maximum likelihood method and the Tamura-Nei Model (Tamura & Nei, 1993) with 1,000 bootstrap pseudo-replicates. For the Dikarya tree there were a total of 98 sequences and a total of 412 positions in the final data set, for the Chytrid and basal tree there were a total of 120 sequences and 442 positions in the final data set. Initial trees for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Broad taxonomic groups on the phylogenies were labelled based on latest phylogenetic classifications in latest version of the UNITE database (Kõljalg et al., 2005), apart from assignment of the Cryptomycota OTU clades which was based on Bass et al. (2018) and novel Chytrid clades (Richards et al., 2015).

3 | RESULTS

3.1 | Occurrence and characterization of plastics

We found a total of 449 pieces of plastic (371 from WSA and 78 from the AP (Lacerda et al., 2019), and from that total, 58 plastics were randomly chosen for metabarcoding analysis, of which 32 were from WSA and 26 from AP. These comprised 25 microplastics (17 plastics in WSA and eight plastics from AP, size range 1–5 mm) and 33 mesoplastic (15 plastics in WSA and 18 plastics from AP, size range 5–74 mm). Each item was classified according to shape as fragment (19 in WSA and 13 in AP), line (11 in WSA and nine in AP), and sphere (two in WSA and four in AP). Polymer composition was determined for plastic pieces selected randomly, 15 from WSA and 13 from AP, with a diverse group of different polymers being recovered (Table 1). Present at both locations were Polyethylene (PE), Polyamide (PA), Polyurethane (PU), Polypropylene (PP), Polystyrene (PS) and present only in samples from WSA was Cellulose Acetate (CA).

3.2 | High-throughput sequencing of plastic biofilms

PCR amplification and sequence library generation success was variable between markers. The ITS2 sequence data set was comprised of 32 samples from the western South Atlantic (WSA) and 26 from the Antarctic Peninsula (AP), with fungi representing 60% and 80% of all reads from the total data set from WSA and AP, respectively. The ITS2 data set had a total of 135 fungal OTUs, with 122 detected in WSA and 97 in AP (Table 2). The 18S V4 data set had 45 samples, 28 from WSA and 17 from AP, with fungi representing 2.2% of the eukaryote data set of the WSA and 4.3% of the AP. 18S V4 detected a total of 59 fungi OTUs, with 55 in WSA and 24 in AP (Table 2). The 18S V9 marker generated data for all 58 plastic samples, 32 from WSA and 26 from AP, and fungi represented respectively 2.9% and 5.6% of all reads. The 18S V9 data set had a total of 110 fungi OTUs, with 76 detected in WSA and 78 detected in AP (Table 2).

## 3.3 | Taxonomic composition (order level) of fungi in the Plastisphere

Across all the marker genes a total of 64 different fungal orders were associated with plastics, with 32 being shared between the two main sampling locations, 21 exclusive to WSA, and 11 unique to AP (Figure 2). The ITS2 data set detected a total of 34 orders, with differences in occurrence depending on the location. For instance, orders Spizellomycetales (Chytridiomycota), Tubeufiales (Ascomycota), Gloeophyllales, Corticiales, Trechisporales and Russulales (Basidiomycota), and Diversisporales (Mucoromycota) were detected only in plastic samples from WSA, whereas Botryosphaeriales, Ophiostomatales and Myrmecridiales (Ascomycota), Ustilaginales (Basidiomycota), and Blastocladiales (Blastocladiomycota) were present only in samples from AP. The 18S V4 detected 25 fungi orders, with Lobulomycetales and Chytridiales (Chytridiomycota), Glomerales (Mucoromycota), Rozellida and LKM11 (Cryptomycota) detected only in WSA and only by the 18S rRNA V4 marker.

Eurotiales and Helotiales were the most frequent orders detected with the ITS2 marker in AP, each present in 23 samples. In WSA, Pleosporales was the most frequent and abundant order, present in 31 plastic pieces, followed by Eurotiales (29 plastics), and Wallemiales (23 plastics) (Figure 3; Data Set S1).

Saccharomycetales was the most frequently found order (7 plastics) within the 18S V4 data set from AP samples. In waters around

TABLE 1 Percentage of plastics samples at the sea surface of the Western South Atlantic (WSA) and around the Antarctic Peninsula (AP), according to their polymer composition

Location	Polymer						Size		Shape		
	PE	PA	PU	PP	PS	CA	Micro	Meso	Fragment	Line	Sphere
WSA	20%	40%	7%	13%	13%	7%	31%	69%	50%	53%	15%
AP	31%	23%	23%	8%	15%	-	53%	47%	56%	43%	6%

Abbreviations: PE, polyethylene; PA, polyamide; PU, polyurethane; PP, polypropylene; PS, polystyrene; CA, cellulose acetate; size: Micro, microplastics < 5 mm; Meso, mesoplastic 5–200 mm; shape: fragment, line, and sphere.

TABLE 2 Number of sequence reads, total number of OTUs and unique and shared OTUs in amplicon sequence libraries for ITS2, 18SV4 and 18SV9 markers amplified from DNA extracted from plastics sampled at the western South Atlantic (WSA) and Antarctic Peninsula (AP)

Marker	Number of fungi reads		Total number of fungi OTUs		Shared OTUs	Unique OTUs	
	WSA	AP	WSA	AP		WSA	AP
ITS	1,062,118	729,369	122	97	84	38	13
V4	9,586	9,683	55	24	20	35	4
V9	43,183	20,836	76	78	44	32	34

(a)

(b)
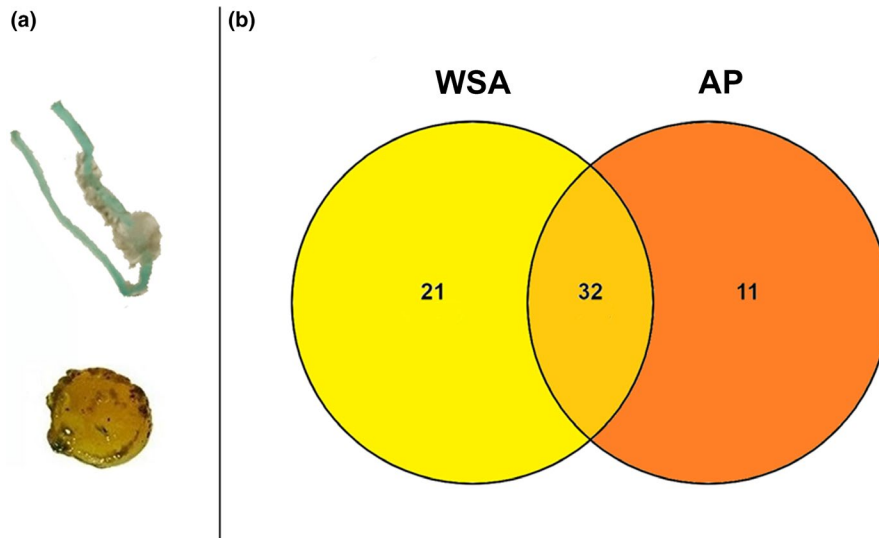
FIGURE 2 (a) Biofilm on the surface of floating marine plastics: nylon line (top) and pellet (bottom); (b) shared and exclusive taxonomic orders of fungi in the Plastisphere of the western South Atlantic (WSA) and Antarctic Peninsula (AP), identified through multiprofile analyses of combined rRNA ITS2 and 18S V4 and V9 regions

AP, the 18S V4 found only seven orders with a frequency of occurrence in two or more samples (Eurotiales, Saccharomycetales, Agaricostilbales, Aphelidea, Rozellida, Rhizophydiales and Zoopagales), but all were present in less than ten samples. In WSA the 18S V4 data set identified more orders than in AP, with a higher frequency of Eurotiales (10 plastics) and Agaricostilbales (seven plastics), followed by Agaricales and Mortierellales (six plastics) (Figure 3).

The 18S V9 data set contained sequences from 43 orders of fungi. The most frequent fungi orders associated with plastics in 18S V9 from waters of the WSA and AP were Eurotiales (23 plastics WSA, and 28 plastics AP), Saccharomycetales, Agaricostilbales, Capnodiales, Aphelidea, and Wallemiales (Figure 3). Both the 18S V4 and V9 were able to detect a higher number of Chytridiomycota taxa, as well as other groups that were not observed not all with ITS2 marker, such as Cryptomycota, Zoopagomycota, Aphelidomycota, and Blastocladiomycota (Figure 3).

3.4 | Comparison of fungal OTU richness and community composition per plastic fragment between regions

The number of fungal taxa associated with each plastic fragment was highly variable in both study regions. In WSA, ranges were 7–23 OTUS for ITS2 (average 13.8 ± 4.1 SE); 1–13 OTUs for 18S V4 (average 4.5 ± 2.9 SE); and 2–25 OTUs for 18S V9 (average 12 ± 5.4 SE). In AP, the range was 7–20 fungal OTUs for ITS2 (average 13.6 ± 2.7

SE); 1–12 OTUs for 18S V4 (average 2.4 ± 3.0 SE); and 3–28 OTUs for 18S V9 (average 9.5 ± 7.0 SE) (Figure 4). Overall, the fungal specific ITS2 detected a higher number of OTUs per plastic fragment than the general 18S V4 and V9 regions. For the 18S V4 marker there was higher number of OTUs per plastic fragment in the WSA than in the AP (Kruskal-Wallis, p = .01). There were no differences in richness between plastics of difference size, shape or polymer type either within each location or the total data set. We also saw no significant correlation between fungal OTU richness and size of plastic fragments. Fungal community composition was highly variable between individual plastic fragments and many samples had low richness, therefore we did not find significant difference in the community composition of fungi between plastics of distinct size classes (micro and mesoplastic), shapes (line, sphere and fragment), polymer composition sampling station or broad location (PERMANOVA, p > .05) (Figure 5 and Figure S3).

3.5 | Phylogeny and potential function of the most frequent fungal OTUs within the plastisphere of the WSA and AP

Aspergillus was the most frequent or second most frequent OTU associated with plastics from WSA and AP in all marker gene data sets (Table 3). Phylogenetic assignment of OTUs matching *Aspergillus* to species level was variable between marker genes: for example, for the WSA ITS2 the most frequent OTU match with 100% similarity
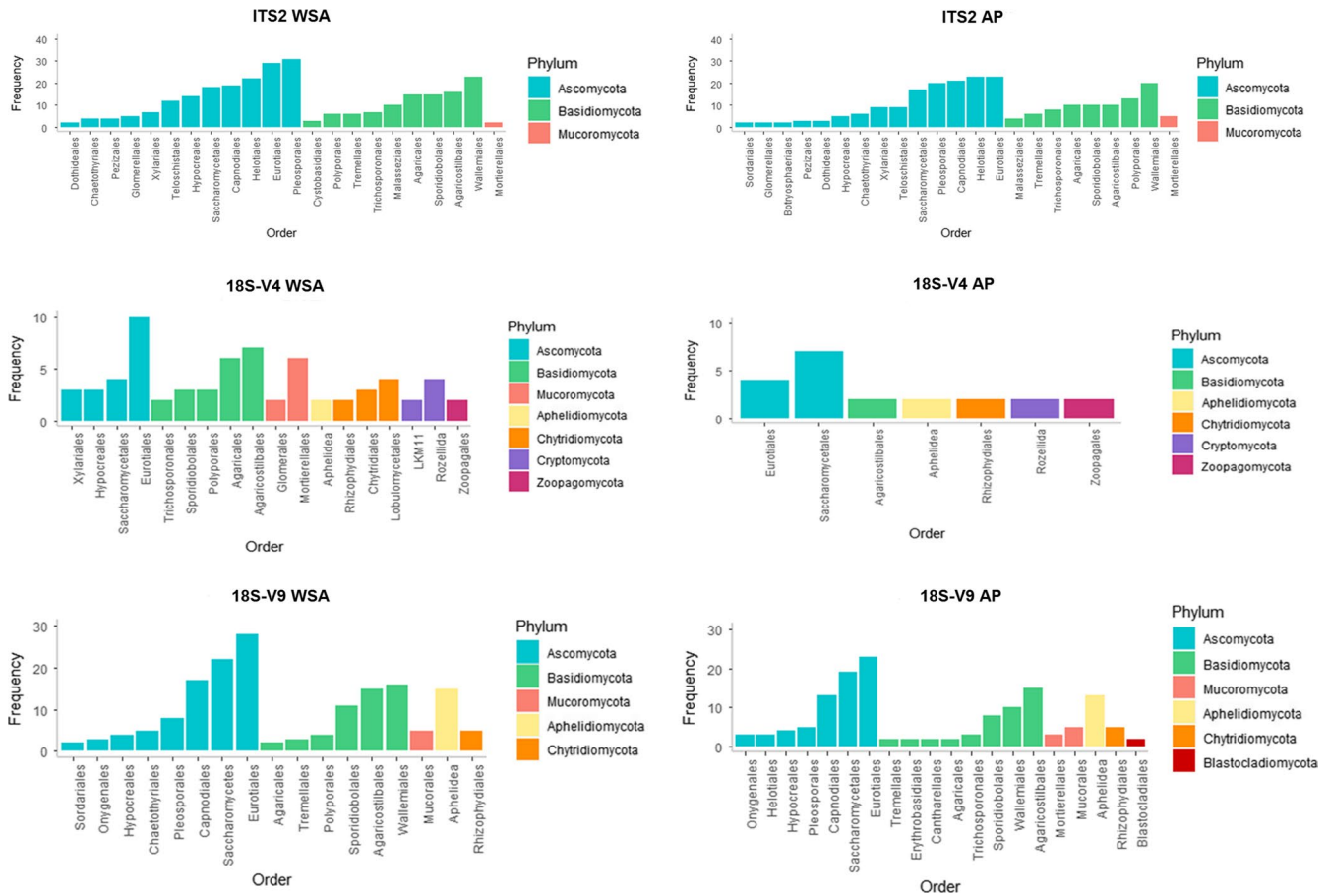
FIGURE 3 Frequency of occurrence of fungal taxonomic orders identified on floating marine plastics from the western South Atlantic and (WSA) and Antarctic Peninsula (AP) detected using metabarcoding with the ITS2 region, 18S V4 and 18S V9 rRNA markers

was *A. vitricola*, isolated from masonry or artworks. However, for 18S V4, the OTU with 100% match was *A. restricus* from the deep sea, and for the 18S V9 the OTU with 100% match was *A. wentii*, isolated from dried marine fish. Several of the most frequent OTUs were closely matched to isolates from saline or low water activity environments. For instance, in the 18S V9 data set we observed the OTU Wallemiales_OTU44, with 100% match to *Wallemia mellicola* from hypersaline waters, and the OTU Agaricostilbales_OTU62 that was 100% matched to *Sterigmatomyces halophilus*, which has been described from multiple marine environments (Table 3). Frequently detected OTUs were also found to have high similarity to some isolates associated with marine flora and fauna, such as Capnodiales_OTU10 in the ITS2 data set which had a high match to a *Cladosporium* sp. isolate from a soft coral, and Capnodiales_OTU97 which was closely matched to a *Cladosporium* sp. isolate from the seaweed *Fucus* sp. (Table 3). Several of the same most frequents OTUs appeared in both the WSA and AP, such as those with high similarity to *Aspergillus* spp., *Cladosporium* spp. and *Wallemia* spp. Many of the OTUs had high similarity to sequences reported in environmental molecular surveys from marine/freshwater, marine sediments, deep sea, sewage and wastewater (Table 3).

The majority of functional assignments using FUNGuild for the top five most frequent OTUs from each location and each marker

were Probable/Highly Probable Saprotrophs, with a few OTUs being assigned uncertain function or being absent in the current taxa database (Table 3). Looking across the whole data set, the majority of OTUs were likely to be Saprotrophs, with some being assigned as Possible/Probable Animal and Plant Pathogens (Data Set S1).

Phylogenetic analysis of 18S V4 OTUs (Figures S1 and S2) revealed highly phylogenetically diverse groups of both Dikarya and Chytrids and basal fungal groups. Almost all taxa in the Chytrid tree (Figure S2) showed no close matches to previously described isolates, although several showed closer relationships to sequences detected in environmental surveys of both marine and freshwater locations. Many of the OTUs clustered with environmental clades of sequences with currently no cultured representatives, i.e., those in the expanded Microsporidia clade. Many of the Dikarya OTUs (Figure S2) were closely affiliated with cultured representations and had high phylogenetic similarity to many previously described taxa from a diverse range of environments.

4 | DISCUSSION

This study is the first to focus on fungi associated plastics in the Southern Hemisphere, as well as the first to evaluate such fungi using
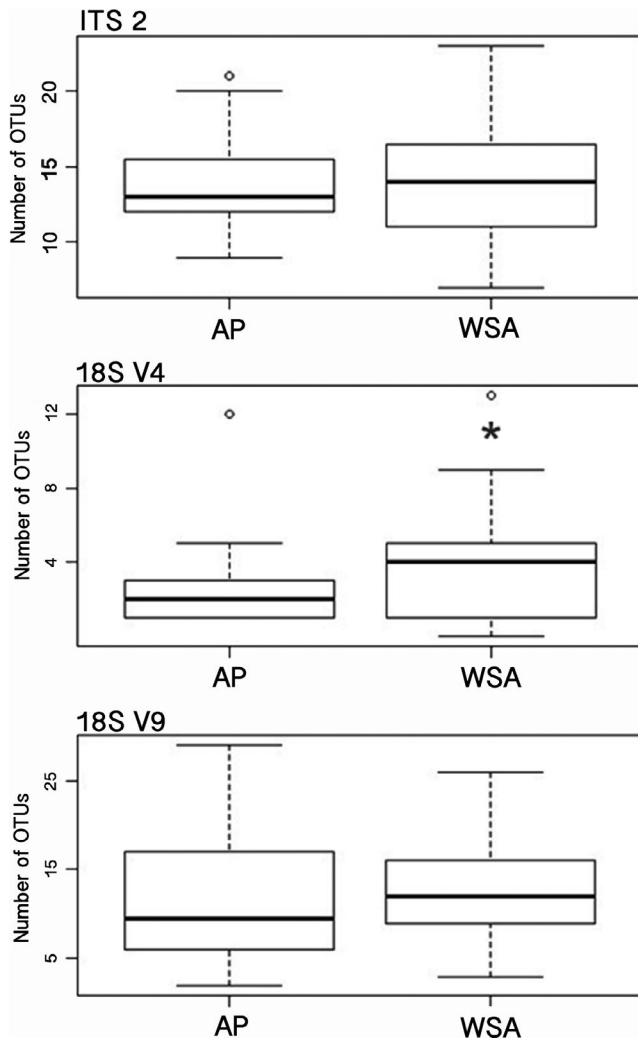
FIGURE 4 Mean number of observed OTUs per plastic sample from the western South Atlantic (WSA) and Antarctic Peninsula (AP), obtained from rarefied ITS2 (1,000 sequence/sample), 18S V4 (300 sequences/sample) and 18S V9 (500 sequences/sample) amplicon sequence libraries. *denotes statistical significant difference (Kruskal-Wallis, $p < .01$)

molecular techniques within coastal and oceanic regions of WSA and AP. It also takes a different approach to studies looking at Eukaryotic communities associated with plastics, by sampling micro and meso-plastics from the environment instead of conducting colonisation experiments (Kettner et al., 2017, 2019; Oberbeckmann et al., 2016) our approach has previously been used for bacteria (Amaral-Zettler et al., 2015), but never for fungi. The approach identified a range of plastic polymer compositions, sizes and shapes, which were similar in composition and characterization to those found in other regions of the Oceans (Debroas et al., 2017; Eriksen et al., 2014). Despite, on average, manta trawls being for longer lengths of time in AP than WSA we detected a lower number of plastic fragments in AP. We detected fungal taxa in our data set that have never been reported within the marine Plastisphere, such as Aphelidomycota, Zoopagomycota, Mucoromycota and Blastocladiomycota. Additionally, this is the first report of Cryptomycota/Microsporidia on open ocean plastics,

which had previously been described only on plastic samples from coastal waters (Kettner et al., 2017). In agreement with studies in the North Sea (De Tender et al., 2017; Oberbeckmann et al., 2016), Ascomycota and Basidiomycota were shown by all markers to be highly frequent plastic colonisers in both sampling sites. However, other studies in coastal waters of the North (Kirstein et al., 2018) and Baltic (Kettner et al., 2017) Seas showed that Chytridiomycota were the most abundant fungal group within plastic biofilms. In our data set, Chytrids were detected, particularly from samples from the AP, but had a low frequency despite often having a high relative abundance in samples where they were present. In Arctic systems Chytrids have been reported as the most abundant fungi group in ice cores (Hassett, Ducluzeau, & Collins, 2017; Hassett & Gradinger, 2016) and seawater (Comeau, Vincent, Bernier, & Lovejoy, 2016), A diversity of Chytrids and other basal fungi have been found in lakes on the Antarctic continent (Rojas-Jimenez et al., 2017), and in Antarctica soils (Cox, Newsham, & Robinson, 2019) as well as on organic particles in the Scotia sea (Duret et al., 2020). Our study demonstrated the benefit of using a combination of marker genes for epiplastic fungi. Not only did this approach provide a greater overall detection of fungal groups, but for some taxa it highlighted differences in the taxonomic resolution between markers: for example, the most frequently encountered *Aspergillus* OTU in each data set, which showed similarity to terrestrial isolates using ITS2 but similarity to marine isolates using 18S. Our 18S primers were general and while this did limit numbers of fungal reads they did still detect many groups and provided better resolution than ITS2. Fungal specific 18S primers could be used to representation of fungi but these may have difficulties detecting all fungal groups (Banos et al., 2018). Our study further highlights there is critical limitation in terms of detection and accurate classification of marine fungi (Banos et al., 2018; Godinho et al., 2013; Richards et al., 2012).

We did not observe distinct geographic differences at the community level for epiplastic fungi between the WSA and AP. This is contrary to patterns previously observed for bacterial communities (Amaral-Zettler et al., 2015) in the Atlantic and Pacific and eukaryotic communities in North and Baltic Seas (Oberbeckmann et al., 2014). We did not observe any measurable differences in community composition in relation to characterisation of the plastics themselves, again this is contrary to studies which have looked at colonisation by Bacteria on different polymers (Oberbeckmann et al., 2016) and those that have looked at Eukaryotic colonisation on different polymer types in environmental samples, although previous studies on eukaryotes have only detected a small number fungal OTUs (Debros et al., 2017). For fungi specifically, a colonisation experiment in the North Sea showed highly variable patterns in fungal communities between a harbour and a coastal site (De Tender et al., 2017). Microplastic-associated bacterial communities may rapidly adapt their composition to changing environments as they move over long distances (Oberbeckmann & Labrenz, 2020). We suggest that the same does not happen with plastic-associated fungi from WSA and AP. Many of the fungal taxa found on the plastics were found in both broad regions, e.g., the genera *Aspergillus*
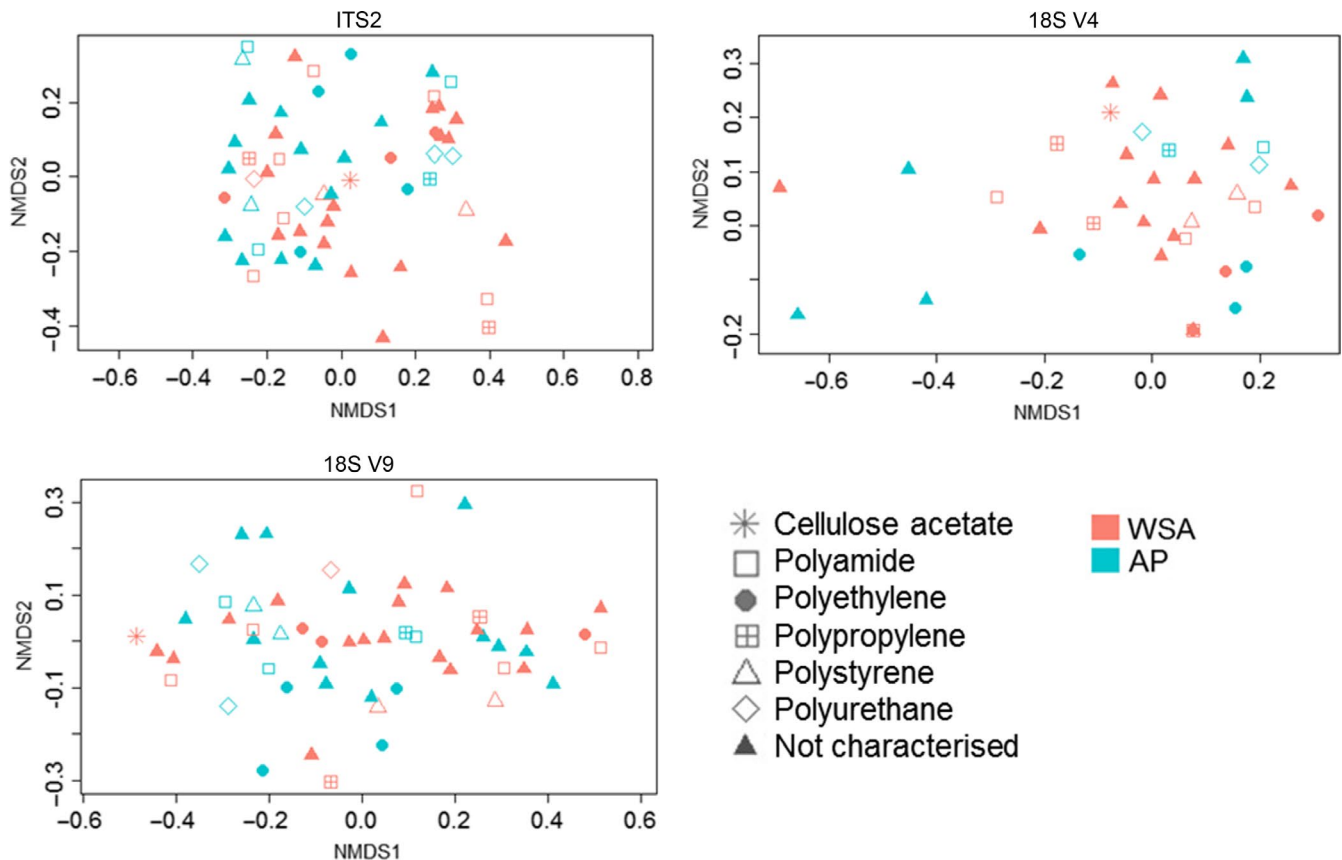
FIGURE 5 Nonmetric multidimensional scaling (NMDS) ordination of jaccard dissimilarity matrices generated from Fungi OTU tables, that were produced using multi-marker DNA metabarcoding (ITS2, 18S V4 and V9 regions) of plastic pollution samples from the Western South Atlantic (WSA) and Antarctic Peninsula (AP). Colours represent sampling location (AP or WSA) and marker shape represents polymer type, if characterized. Stress values ITS2 = 0.23, 18S V4 = 0.15, and 18S V9 = 0.19)

and *Cladosporium* and the order Pleosporales, and indeed these genera are cosmopolitan within the marine environment and found in many locations (Hassett, Vonnahme, Peng, Jones, & Heuzé, 2019; Richards et al., 2012; Rosa, 2019). Some of the fungi OTUs identified in the specific and general data sets were common to both WSA and AP, e.g., *Sterigmatomyces halophilus*, *Aspergillus versicolor* and *Cladosporium* sp. All of these OTUs have been previously reported in other marine environments (Bovio et al., 2017; Raghukumar, 2017), with *S. halophilus* being one of the most abundant and frequent OTUs in both regions. The waters of the AP are significantly colder than those of WSA, yet it seems that there is no temperature barrier to many of the plastic associated fungal taxa. Many of the fungi we found have been detected in Arctic systems using molecular methods (Hassett, Borrego, et al., 2019; Hassett & Gradinger, 2016) and some groups have been detected previously in Antarctic waters using isolation and culturing (Gonçalves et al., 2017). We also found a range of taxa reported from the marine environment but not previously found on plastics. For example, an OTU matching *Meyerozyma guilliermondii* was found in high abundance and frequency in plastics from the AP; this fungal taxa has been previously reported associated with invertebrate species (Duarte et al., 2013; Godinho et al., 2019) and macroalgae (Godinho et al., 2013).

We did not measure seawater "free-living" communities to compare to plastic associates as previous studies have done for other microbial groups, although in all cases they have all shown clear differences between plastic associates and seawater communities (Debroas et al., 2017; Kettner et al., 2019; Oberbeckmann, Kreikemeyer, Labrenz, & Harrison, 2018). In marine environments fungi appear to be more abundant in > 3 up to 90 μm size fractions (Gutiérrez, Pantoja, Tejos, & Quiñones, 2011; Richards et al., 2015), suggesting they themselves are large or are particle associated. It is likely that most saprotrophic fungi would be attached/associated with some form of particle, either organic (i.e., transparent exopolymer particles (Cunliffe et al., 2017)) or synthetic such as plastic, as this is where they can readily access carbon for growth. This means that without detailed size fractionation it would be difficult to separate true free-living fungal communities in seawater. In our study while we did see some common fungal taxa across plastics, at individual sampling stations plastic associated fungal communities were different suggesting we are not just detecting free-living taxa, which in seawater would be relatively homogenous at each individual sampling station.

The transport of plastics by oceanic currents could be an explanation for the similarity of many fungal taxa detected between the WSA and AP. The WSA is influenced by the Malvinas (Falkland)

TABLE 3 Top five most frequent fungi OTUs from ITS2 and 18S V4 and V9 amplicon libraries obtained from marine plastic biofilms from the western South Atlantic (WSA) and Antarctic Peninsula (AP)

Order and OTU #	Guild	Environmental sample			Cultured isolate				Fre.	Abun.
		Source	ID %	Accession	Species	ID %	Accession	Source		
ITS										
WSA										
Eurotiales_OTU6	S	Seagrass rhizosphere	100	MH364700.1	Aspergillus vitricola	100	MK367420.1	Cathedral Fresco	25	4%
Wallemiales_OTU1	S	Marine sediment	100	GU370753.1	Wallemia mellicola	98	FJ770080.1	Sponge parasite	23	14%
Agaricostilbales_OTU2	S	Seawater	100	KU163776.1	Sterigmatomyces halophilus	100	KY105557.1	Soil	13	12%
Capnodiales_OTU10	PSSY	Marine sediment	100	MH368301.1	Cladosporium cladosporioides	100	MH244422.1	Coral associate	13	6%
Sporidiobolales_OTU13	PS	Seagrass	100	MH364421.1	Rhodotorula mucilaginosa	100	MH231247.1	Sea water	13	<1%
AP										
Wallemiales_OTU1	S	Marine sediment	100	GU370753.1	Wallemia mellicola	98	FJ770080.1	Sponge parasite	19	9%
Eurotiales_OTU6	S	Seagrass rhizosphere	100	MH364700.1	Aspergillus vitricola	100	MK367420.1	Cathedral Fresco	17	9%
Capnodiales_OTU10	PSSY	Marine sediment	100	MH368301.1	Cladosporium cladosporioides	100	MH244422.1	Coral associate	15	3%
Agaricostilbales_OTU2	S	Seawater	100	KU163776.1	Sterigmatomyces halophilus	100	KY105557.1	Soil	10	10%
Saccharomycetales_OTU5	S	Hydrothermal vent	100	KX430947.1	Meyerozyma guilliermondi	100	KY401420.1	Brackish water	10	14%
18S rRNA V4										
WSA										
Eurotiales_OTU181	S	Seafloor	100	KR072832.1	Aspergillus restrictus	100	EU723495.1	Deep sea	10	1%
Agaricostilbales_OTU62	S	Acid hot spring	98	EF682445.1	Sterigmatomyces halophilus	100	NG062686.1	Anoxic seawater	6	9%
Mortierellales_OTU123	NA	Marine sediment	98	JX110992.1	Mortierella hyalina	99	JQ040259.1	Plant root	6	4%
Agaricales_OTU67	PSY	Seawater	98	GU824934.1	Pachylepyrium sp.	98	HQ832429.1	Coastal grassland	6	5%
Hypocreales_OTU95	S	Soil	99	AY969172	Trichoderma amazonicum	99	NG_062836.1	Plant Endophyte	3	3%
AP										
Saccharomycetales_OTU46	S	Deep-sea	100	JF308274.1	Meyerozyma guilliermondii	100	MK355207.1	Compost	6	10%
Eurotiales_OTU181	S	Seafloor	100	KR072832.1	Aspergillus restrictus	100	EU723495.1	Deep sea	4	<1%
Sporidiobolales_OTU130	S	Antarctic ocean	100	MK003687.1	Rhodotorula evergladensis	100	NG_063017	Seawater	2	1%
Zoopagales_OTU79	NA	Sulfidic spring	87	KT072153.1	Zoopage sp.	90	MG920182.1	Rotifer parasite	2	2%
Agaricostilbales_OTU62	S	Acid hot spring	98	EF682445.1	Sterigmatomyces halophilus	100	NG062686.1	Air	2	<1%
18S rRNA V9										
WSA										
Eurotiales_OTU19	S	Marine sediment	100	GU474197.1	Aspergillus wentii	100	AB002063	Dried fish	27	44%
Capnodiales_OTU97	S	Soil	99	EU490070.1	Cladosporium sp.	100	MH102092.1	Seaweed	17	2%

(Continues)

TABLE 3 (Continued)

Order and OTU #	Guild	Environmental sample			Cultured isolate					
Wallemiales_OTU44	S	Seawater	100	JF826393.1	Wallemia mellicola	100	AY741380.1	Hypersaline water	16	13%
Eurotiales_OTU489	S	Antarctic snow	99	KR131435.1	Aspergillus penicillioides	99	AF548066	Air	16	3%
Agaricostilbales_OTU42	S	Seawater	93	FJ153656.1	Sterigmatomyces halophilus	100	NG062686.1	Anoxic seawater	15	12%
AP										
Eurotiales_OTU19	S	Marine sediment	100	GU474197.1	Aspergillus wentii	100	AB002063	Dried fish	21	38%
Agaricostilbales_OTU42	S	Sea water	93	FJ153656.1	Sterigmatomyces halophilus	100	NG062686.1	Anoxic seawater	15	6%
Capnodiales_OTU97	S	Savanna soil	99	EU490070.1	Cladosporium sp.	100	MH102092.1	Seaweed	13	2%
Saccharomycetales_OTU213	S	Activated sludge	100	AB901846.1	Geotrichum sp.	100	MF381141.1	Freshwater	10	4%
Wallemiales_OTU44	S	Sea water	100	JF826393.1	Wallemia mellicola	100	AY741380.1	Hypersaline water	10	5%

Note: Order/OTU number: Guild: the potential trophic guilds of each OTU. S, saprotroph; P, pathotroph; SY, symbiotroph; PSY, pathotroph-saprotroph-symbiotroph; PS, pathotroph-saprotroph; PSY, pathotroph-symbiotroph; NA, not assigned; comparisons with sequenced environmental and cultured isolates; Accession number, similarity (ID %), and source/highlights); Freq, the frequency of occurrence of the OTUs on plastics; Abun, the relative abundance of the OTU in the total data set (%).

current, which originates in the Southern Ocean and flows northwards, reaching the studied region mainly during early autumn (when samples were collected) and winter (Matano et al., 2010; Zavialov, Ghisolfi, & Garcia, 1998). Also, near-surface oceanic currents act in the transport of plastics in the opposite direction, from South Atlantic to the Southern Ocean (Wichmann et al., 2019). Therefore, plastics and their associated organisms could be transported between areas. Many fungal taxa, particularly benthic fungi, may have their dispersal capacity amplified by attachment to floating plastics. The transport of spores between locations mediated by plastics could result in bioinvasions (Amaral-Zettler et al., 2015; Barnes, 2002; Carlton et al., 2017) and the ecological impacts could be irreparable.

Colonisation patterns of plastics will be variable depending on which organisms are present at the moment in which plastics entered the marine ecosystem, particularly at coastal sites. Considering that many aquatic and terrestrial fungi are able to cope with increased salinity (Kettner et al., 2017), plastics from freshwater and terrestrial environments that reach the open ocean may be a dispersal vector of fungal groups. This may explain why there was a higher number of fungal orders detected on plastics from WSA than those from AP, but also the why the mean number of OTUs per plastic piece were higher in WSA than AP for the 18S V4 data. With a potential mix of terrestrial/freshwater and marine taxa, increasing the richness of plastic associated fungi in WSA. To support this, several taxa from terrestrial and freshwater environments (i.e *Gibellula* sp., *Skeletocutis diluta*, *Glomus* sp., *Cyberlindnera jadinii*, unclassified Russulales) were found in WSA from the coastal stations of WSA which were very close to the mouth of large fluvial drainage basins (station 1, station 7). It was surprising to find mycorrhizal taxa from the Glomeromycotina and Russulales associated with plastics in the marine environment but both groups have been detected in coastal ecosystems such as mangroves and saltmarshes (Alzarhani et al., 2019; Luis et al., 2019) and also within coastal plankton samples (Taylor & Cunliffe, 2016). Phylogenetic analysis of the Glomeromycotina OTUs identified them as associates with several different tree species. Therefore, it is highly likely this was detection of spores that attached to the plastics, this occurred either within the terrestrial environment and the plastics were washed into marine systems or spores in coastal soils or river banks were washed into coastal waters and attached to floating plastics.

Detection of spores raises an important question about the activity of several of the fungal taxa associated with our plastics. However, given that the majority of the groups have been reported in marine environments world-wide (Hassett, Vonnahme, et al., 2019; Richards et al., 2015; Taylor & Cunliffe, 2016) and several studies have used RNA based approaches (as a marker of active taxa RNA is degraded more rapidly than DNA and is continually produced by activity metabolising cells) and detected similar taxa in our study it is likely that majority of taxa are indeed active (Orsi, Biddle, & Edgcomb, 2013; Richards et al., 2015). In marine plastic pollution research, further work should aim to address the issue of activity with RNA based techniques and microscopy.

Another novelty of this work is the assignment of potential trophic guilds using FUNGuild (Nguyen et al., 2016). The majority of OTUs classified were saprotrophs. Saprotrophic fungi are key regulators of nutrient cycles in terrestrial systems and it is thought this is true in marine ecosystems (Amend et al., 2019; Cunliffe et al., 2017; Grossart et al., 2019). It has been suggested that early biofilm colonizers on plastics, such as Bacteria, can be attracted not to the plastic's surface, but to the biofilm that could increase their access to nutrients (Oberbeckmann & Labrenz, 2020). The same could apply for many saprotrophic fungi: once the early colonizers are established, secondary colonizers start to develop a diverse community living in association with other organisms and living off their exudates and organic matter. Metagenomes may identify genes and enzymes from fungi involved in these processes (Bryant et al., 2016), but due to lower biomass of fungi compared bacteria or metazoa in epiplastic, analysis of function in fungi in plastic samples may be challenging. In oceanic systems sequence reads assigned to Fungi in metagenomes only make up around 1%–2% of the total sequence reads (Morales, Biswas, Herndl, & Baltar, 2019).

Several OTUs in both WSA and AP that closely matched isolates from marine animals and plants, such as OTUs of genera *Cladosporium*, *Wallemia Aspergillus* and *Alternaria*, which have been found association with corals, sponges, macroalgae and marine plants (Ein-Gil et al., 2009; Godinho et al., 2013; Yarden, 2014). *Zoopage* sp., present in both WSA and AP samples, have been described as parasites of amoebas, nematodes and rotifers (Spatafora et al., 2016). Many other eukaryotic groups were detected in our data and this supports the suggestion that some fungi groups could be living associated with these organisms. OTUs within Chytridiomycota, Rozellomycota, Aphelidomycota, Cryptomycota which have been shown to be parasites of several algal groups (Gerphagnon, Colombet, Latour, & Sime-Ngando, 2017; Kagami, De Bruin, Ibelings, & Van Donk, 2007) including diatoms, were also detected. A number of OTUs were found matching undescribed Aphelidiomycota and Rozellomycota taxa (see Figure S2), which are parasites of a range of organisms (Karpov et al., 2014; Letcher & Powell, 2019) and could be parasitizing the dense microalgal biofilms on the surface of plastics (Lacerda et al., 2019; Oberbeckmann et al., 2016). Phylogenies also revealed taxa with affinities within the expanded clade of microsporidia, these obligate parasites (Bass et al., 2018) are likely to be parasitizing protists and metazoans within the biofilms.

The role of marine plastics as vectors for disease is still poorly understood (Dussud et al., 2018; Kettner et al., 2019; Masó, Garcés, Pagès, & Camp, 2007; Oberbeckmann et al., 2018). Considering the small size of some of our sampled plastics (<5mm), they could be ingested by organisms from low trophic levels (Desforges, Galbraith, & Ross, 2015; Setälä, Fleming-Lehtinen, & Lehtiniemi, 2014) to apex predators (Lusher et al., 2015; Wilcox, Mallos, Leonard, Rodriguez, & Denise, 2016). Several of the fungi taxa were identified as been possible or probable pathotrophs such as *Acremonium* sp. that have been described as parasites of the brown algae *Fucus serratus* (Zuccaro et al., 2014) and some animal taxa.

Many of the taxa identified may have the ability to degrade plastics, such as members of the genus *Aspergillus*, which are able to degrade plastic polymers such as polyvinyl chloride, polyethylene (Shah, Hasan, Hameed, & Ahmed, 2008) and high and low density polyethylene (Gajendiran, Krishnamoorthy, & Abraham, 2016; Pramila & Ramesh, 2017; Sangeetha Devi et al., 2015). *Cladosporium*, found in our work and previously on plastic biofilms, have also been shown to degrade plastics (Brunner, Fischer, Rüthi, Stierli, & Frey, 2018; Shah et al., 2008). We also found unclassified members of Pleosporales in samples from WSA and AP, some members of this order are able to degrade polyurethane (Russell et al., 2011). Unfortunately from our marker gene metabarcoding alone we cannot know whether the fungi we detected are able to degrade plastics and using it as a substrate for growth. Certainly, the relatively destructive method of processing suggests that many of the fungi may be tightly bound to the plastic surface, with potential for mycelial penetration of the plastics. Most evidence of plastic biodegradation by fungi is obtained under laboratory conditions, further work should aim to isolate fungi from marine plastics to characterise their degradation potential.

In conclusion, this study reports a wide range of phylogenetically and functionally diverse fungal taxa in coastal and oceanic waters of the Plastisphere of the western South Atlantic and Antarctic Peninsula, with some groups shared and some unique to each region. Our findings highlight the importance of combining molecular markers for a more robust profiling and characterization of fungal diversity. We report fungi groups that had not yet been described living on marine plastics and observed a highly variable phylogenetic assemblage of predominantly saprotrophic taxa. Considering the high diversity of fungi detected here and in previous studies of the Plastisphere, there is an urgent need to further investigate the functions and ecology of fungi living on marine plastics, as well as their potential interactions and impacts on other organisms and environments.

AUTHOR CONTRIBUTIONS

DATA AVAILABILITY STATEMENT

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.