Partitioning of fungal assemblages across different marine habitats

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Summary

Fungi are a highly diverse group of microbes that fundamentally influence the biogeochemistry of the biosphere, but we currently know little about the diversity and distribution of fungi in aquatic habitats. Here we describe shifts in marine fungal community composition across different marine habitats, using targeted pyrosequencing of the fungal ITS region. Our results demonstrate strong partitioning of fungal community composition between estuarine, coastal and oceanic samples, with each habitat hosting discrete communities that are controlled by patterns in salinity, temperature, oxygen and nutrients. While estuarine habitats comprised a significant proportion of fungal groups often found in terrestrial habitats, the open ocean sites were dominated by previously unidentified groups. The patterns observed here indicate that fungi are potentially a significant, although largely overlooked, feature of the ocean's microbiota, but greater efforts to characterise marine species are required before the full ecological and biogeochemical importance of marine fungi can be ascertained.

Fungi are present within a wide range of marine habitats (e.g. Jones & Pang, 2012, Manohar & Raghukumar 2013) and their importance in aquatic ecology, disease, biogeochemical cycling and trophodynamics is rapidly emerging (e.g. Amend *et al*., 2011, Wang *et al*., 2012, Krause *et al*., 2014, Kagami *et al*., 2014). However, relative to terrestrial environments, our understanding of the diversity and spatiotemporal dynamics of fungi inhabiting the ocean is still undeveloped. This is particularly true when compared to the deep understanding of marine bacterial and archaeal dynamics that has been acquired in recent decades (e.g. Karl, 2007). Indeed, we are only just beginning to consider and understand patterns in the phylogenetic composition of fungal communities across different ocean habitats (Manohar & Raghukumar 2013). For example, novel fungal phylotypes and higher fungal diversity were found in coastal waters compared with open ocean environments in twelve samples taken over a range of depths near Hawaii (Gao *et al*., 2010). However, previous work has applied fingerprinting or clone library approaches, *and to our knowledge no study has utilized the increased sequencing depth and resolution provided by next-generation sequencing* applied targeted next-generation sequencing to investigate aquatic fungal communities across multiple marine habitats and across physicochemical gradients. Until we understand the spatiotemporal distribution of specific fungal populations, and describe the diversity of fungi in aquatic habitats as a baseline to begin to identity the most ecological and biogeochemically relevant members of the fungal community, we will not be able to adequately integrate fungi into our general understanding of the microbial ecology of the oceans.

To begin to address this knowledge gap, we examined patterns of fungal community structure across different marine habitats by comparing assemblages from 65 sites, spanning a semi eutrophic urbanized estuary (Sydney Harbour) through to coastal habitats (Australian

84 east coast) and the open South Pacific Ocean (Supporting information Figure S2 & Table
85 S1). Fungal community assemblages, characterised using ITS targeted 454-pyrotagging
86 (Supporting information and figure captions for details), displayed significant shifts in
87 community composition across a spatial gradient spanning estuarine to open ocean
88 conditions (ANOSIM Global R = 0.89 , Sig. < 0.05, Figure 1A; Supporting Figure S3A). A
89 network analysis approach, which groups samples together based on shared operational
90 taxonomic units (OTUs), revealed that each marine environment represents a distinct fungal
91 habitat, with estuarine and open ocean samples forming clearly separated clusters, and
92 coastal samples emerging as a transitional zone between them (Figure 1B, Supporting Figure
93 S3B).
94
95 Across our entire data set, more than 80% of sequences could not be matched to any known
96 phylogenetic group in the UNITE database (Kõljalg *et al*., 2005) using BLAST ($E<10^{-5;}$
97 Altshul *et al*., 1997) (Figure 2). This is perhaps not surprising, given the poor representation
98 of aquatic fungi in public databases, which comprise sequences predominantly derived from
99 soil environments (Kõljalg *et al*., 2005). However, it is notable that the proportion of
100 identifiable fungi varied significantly between environments, with 90% of open ocean OTUs
101 unidentified, while this number dropped to 68% in the estuarine environments.

102 Among the identifiable sequences, strong compositional differences were observed between
103 habitats (Figure 2), confirming the patterns of spatial partitioning observed at the OTU level.
104 Estuarine samples were generally dominated by common soil-inhabiting classes, including
105 hypocreales, helotiales, orbiliales, capnodiales, pleosporales, eurotiales, saccharomycetiales,
106 polyporales, glomerales, agaricales and sordariales. These groups made up 30% of total
107 sequences in estuarine samples, but only 12 % and 10% of sequences in the coastal and open
108 ocean samples, respectively. While most of these groups are often considered as terrestrial or

soil associated fungi, some classes, including the pleosporales and hypocreales, have also been found to occur on the surfaces of aquatic plants such as mangroves (Suetrong *et al*., 2009; Pang *et al*., 2010). In contrast to the estuarine samples, coastal samples were dominated by Chytrids (36%), from the order Rhizophydiales, which were particularly abundant in coastal river plume samples (Figure 2). The Rhizophydiales were also dominant feature (30%) of the identified open ocean samples. Interestingly, this group was much less abundant (0.46%) in the estuarine samples. Rhizophydiales are typically saprotrophs, or parasites of a wide range of organisms including other chytrids, invertebrates, amphibians and phytoplankton (Jones, 1993), and have previously been shown to be associated with phytoplankton blooms (Gerphagnon *et al*. , 2013; Rasconi *et al*., 2012). Existing evidence of chytrids in the marine environments is scarce, with only three lineages previously identified, including species of *Rhizophydium*, *Thalassochytrium* and *Chytridium* (Gleason *et al*., 2011). However recent research suggests that chytrids play an important role in aquatic foodwebs by transferring nutrients between phytoplankton and zooplankton via grazing (Kagami *et al*., 2014). The large representation of this group in the coastal and open ocean assemblages identified here supports an emerging view of the ecological significance of chytrids in the ocean.

There is ongoing debate over the origins of marine fungi, with one hypothesis suggesting that most are of terrestrial origin, either washed into the ocean by rivers or blown in on dust (Jones & Pang, 2012). This may be true for estuarine samples, but our data suggests that only a small proportion of the open ocean fungi have clear taxonomic similarities to terrestrial fungi. Distance based linear modelling indicated that the observed variation in the fungal communities was driven by changes in salinity, temperature, dissolved oxygen, nitrate, silicate and phosphate (Supplementary Figure S1). This observation suggests that aquatic fungi respond to environmental gradients, potentially playing a role in marine nutrient cycles.

However, this relationship between nutrient concentration and fungal community composition requires further research to show that fungi are indeed actively metabolising these nutrients. Other factors that may further influence marine fungal diversity include the availability of substrata for colonization such as particulate organic carbon and plant, animal and algal hosts (Jones, 2000) and these may be factors in our data, particularly in determining the diversity of particle associated taxa in river-plume samples and of potentially phytoplankton associated chytrids. Additionally, other biotic components such as predation and competition may also be responsible for driving patterns in aquatic functional assemblages as for other microorganisms, however this requires further investigation.

Here, we provide new evidence for substantial spatial heterogeneity in the structure of marine fungal communities across different ocean habitats. Estuarine habitats were characterised by the highest proportions of identifiable taxa, with a significant proportion of organisms previously observed in terrestrial environments. This is perhaps indicative of allocthonous inputs of terrestrial fungi into the aquatic environment or the occurrence of a resident coastal/estuarine fungal community that has close phylogenetic links or history with terrestrial populations. The coastal habitat represents an ecotone, where terrestrial, estuarine and "open ocean fungi" co-exist, while the open ocean environment is dominated by a high proportion of previously unidentified taxa that potentially represent a pool of fungi that have specifically evolved to thrive within nutrient poor open ocean conditions. Taken together these patterns indicate that marine fungal communities are shaped by local environmental conditions and are not simply passive colonizers from terrestrial ecosystems, as has previously been proposed (Jones & Pang, 2012). Although the heterogeneous nature of these marine fungi distributions and their relationship to environmental gradients indicate that they are potentially ecologically important members of marine microbial assemblages, given that a

158 significant proportion of OTUs remain unidentified, it is currently challenging to fully
159 decipher the roles that they play in marine environments.

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Figures

Fig 1. (A) Multidimensional scaling of relative OTU abundance (Estuary ●, Coastal ▲ and Ocean ■),and (B) Network analysis of OTU partitioning between samples. Sample clustering represents the degree to which OTUs are shared between samples, with samples grouping closer together if they share more OTUs. Black nodes represent OTUs, coloured nodes represent sample type (red = estuary; green = coastal; blue = open ocean) and edges (lines) connect OTUs to samples in which they occur (coloured by the habitat of the connected sample). Edge weights represent the number of sequences from each OTU that occurred in each sample. OTUs were clustered at 97% identity using UCLUST (Edgar, 2010). Networks were generated using QIIME (Caporaso et al 2010) and visualized using Cytoscape (Shannon et al, 2003). All samples were rarefied to 1082 sequences per sample to equalize sampling depth (details in Supporting information).

Fig.2. Community composition bar graph of OTUs with matches to identified classes in the UNITE database (Kõljalg, 2007). Samples belonging to the Ocean group are designated Tasman Sea (TS), South Tasman Sea (STS) and East Australia Current (EAC). Coastal samples are designated Coast-Harbour (CH), Coastal-River (CR) and Coastal (C). Estuary samples are labelled E. Data was processed using QIIME (Caporaso et al 2010) with OTUs were clustered at 97% identity using UCLUST (Edgar, 2010). Taxonomy was assigned using BLAST ($E<10^{-5}$, Altschul et al, 1997). All samples were rarefied to 1082 sequences per sample to equalize sampling depth (details in Supporting information). Only sequences with matches to the UNITE database at Phylum or Class level are shown here, with the remaining proportions representing sequences for which no annotation beyond "Unidentified (Kingdom_fungi)" was possible.