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Distribution patterns of active fungal communities

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Originality-Significance Statement:

A growing number of studies are making use of RNA sequencing to demonstrate differences in the taxonomy of active and total microbial communities, as characterised using RNA and DNA respectively. Here we make use of isolated and environmentally extreme Antarctic islands to compare the biogeographic characteristics of active and total fungal communities, demonstrating for the first time that endemic fungi are more active in the natural environment than cosmopolitan fungi. Our findings imply that views of soil fungal communities offered by DNA- and RNA-based characterisation may be biased towards particular taxa in a predictable manner.

Summary

Our understanding of the diversity and community dynamics of soil fungi has increased greatly through the use of DNA-based identification. Community characterisation of metabolically active communities *via* RNA sequencing has previously revealed differences between 'active' and 'total' fungal communities, which may be influenced by the persistence of DNA from non-active components. However, it is not known how fungal traits influence their prevalence in these contrasting community profiles. In this study, we co-extracted DNA and RNA from soil collected from three Antarctic islands to test for differences between total and active soil fungal communities. By matching these geographically isolated fungi against a global dataset of soil fungi, we show that widely dispersed taxa are often more abundant in the total community, whilst taxa restricted to Antarctica are more likely to have higher abundance in the active community. In addition, we find that active communities have lower richness, and show a reduction in the abundance of the most dominant fungi, whilst there are consistent differences in the abundances of certain taxonomic groups between the total and active communities. These results suggest that the views of soil fungal communities offered by DNA- and RNA-based characterisation differ in predictable ways.

Introduction

Soil fungi have pivotal roles in terrestrial ecosystems as decomposers, pathogens and partners in mycorrhizal and lichen symbioses. Molecular techniques have substantially increased our knowledge of fungal communities in soils across the globe (Tedersoo *et al.*, 2014), particularly with the onset of next-generation techniques, which preclude the use of a cloning step and have allowed the direct sequencing of soil fungal nucleic acids. Almost all studies carried out to date have relied on the sequencing of soil fungal DNA, which is recalcitrant and relatively long-lived in the environment (Willerslev *et al.*, 2007). As a result, these studies are likely to have sequenced DNA extracted from inactive sources, such as spores, dead hyphae and extracellular DNA (Van der Linde and Haller, 2013), with a recent study estimating that the latter can account for up to 40% of total DNA recovered from soils (Carini *et al.*, 2016). By measuring a soil's 'total' fungal community (*i.e.*, that derived from its DNA profile), it is not possible to distinguish fungi that are active in processes such as decomposition from those that may represent relic or dormant fungal communities, and it is possible that descriptions of richness and relative abundances of taxa are consequently skewed (Carini *et al.*, 2016).

Soil fungal community composition can also be determined through sequencing of the precursor rRNA internal transcribed spacer (ITS) region, a much shorter-lived molecule

65 than DNA, which is present predominantly in metabolically active tissue (Anderson and
66 Parkin, 2007). While this technique has been used in relatively few studies focused on fungi,
67 it has been shown that a soil's 'active' fungal community (*i.e.*, that derived from its RNA
68 profile) may have lower richness than the community measured through DNA-based
69 analyses, and certain taxa may show markedly different abundances (Taylor *et al.*, 2010;
70 Rajala *et al.*, 2011; Baldrian *et al.*, 2012; Van der Linde *et al.*, 2012; Romanowicz *et al.*,
71 2016). Despite this disparity, studies have not yet attempted to determine how fungi
72 possessing different life history traits might be represented in total and active soil
73 communities. If community descriptions based on analyses of DNA are biased towards
74 specific taxa displaying similar traits, our understanding of fungal communities across the
75 globe may be skewed, in a manner analogous to the biases inherent in culture-based
76 community descriptions, which select for generalist species capable of growing on artificial
77 media under laboratory conditions (Bridge and Spooner, 2001).
78 We have previously demonstrated a striking bimodal distribution of range sizes
79 among Antarctic soil fungi (Cox *et al.*, 2016), showing the presence of endemic taxa – which
80 is to be expected, owing to the region's geographical isolation – and a large number of
81 cosmopolitan fungi with global range sizes. Fungi with such wide distributions may be habitat
82 generalists, although the presence of bipolar fungi (occurring at high latitudes in both

hemispheres, but not in between) suggests that at least some are cold-adapted taxa. This disparity in range sizes may be owing to different dispersal strategies, and represents an opportunity to test for the contributions of endemic and cosmopolitan fungi to the total and active fungal communities of geographically isolated maritime Antarctic soils. Gaining a better understanding of the active fungal community of these soils is also imperative, since they are likely to contain significant stocks of old, recalcitrant carbon fractions (Newsham *et al.*, 2018), and decomposition processes in them are thought to be dominated by fungi (Yergeau *et al.*, 2007).

Here, we develop a conceptual framework (Figure 1), in which the relative abundances of fungal taxa in total and active Antarctic soil communities is based on their presence across global sites and in Antarctic soils. We predict that endemic fungi found exclusively in Antarctica will be more abundant in the active community, because of the adaptations of these species to local environmental conditions (Imbert *et al.*, 2011; Berdahl *et al.*, 2015), such as the aridity, low temperatures (<0 °C for c. eight months per annum and annual minima to -40 °C) and wide temperature fluctuations (annual ranges 35–65 °C) encountered in maritime Antarctic soils (Convey *et al.*, 2018). At the other extreme, we anticipate that cosmopolitan fungi identified as occurring in soils across the globe will be relatively less abundant in active Antarctic soil communities, because of their poorer

101 adaptations to local conditions (Büchi and Vuilleumier, 2014). In addition, compared with
102 endemic fungi, cosmopolitan fungi with large range sizes may produce greater numbers of
103 dormant spores containing little RNA relative to DNA (Van der Linde and Haller, 2013).
104 Between these two extremes, we hypothesize that bipolar fungi are likely to produce copious
105 spores capable of dispersal over large distances, replicating the dispersal strategy of
106 generalist species and mirroring the long-distance dispersal of some specialist plant and
107 animal species between suitable habitat patches (Spiegel and Nathan, 2007; Centeno-
108 Cuadros *et al.*, 2011). However, as habitat specialists, these fungi should be capable of
109 active growth and metabolism in Antarctic soils, so on balance may show a more even
110 abundance across the DNA and RNA pools, depending on the relative strength of these two
111 drivers.

112 In the present study, we characterise the active and total fungal communities present
113 in soils from three geographically isolated maritime and sub-Antarctic islands. Fungal
114 community profiles derived from analyses of soil RNA and DNA are compared to explore
115 how they differ in terms of species richness, community evenness, community and
116 taxonomic composition, as well as how the magnitude of these differences changes with soil
117 depth, which has been found to influence the abundance of fungi in numerous habitats
118 (Dickie *et al.*, 2002; Lindahl *et al.*, 2007), and of endemic Archaea in fumarolic soils of

Antarctica (Herbold *et al.*, 2014). By comparison with a global dataset of fungi (Cox *et al.*, 2016), we assign the fungi identified in Antarctic soils as putatively endemic, cosmopolitan or bipolar, to test whether patterns of abundance match those predicted by traits in the conceptual framework shown in Figure 1.

125 **Results**

126 *Sequence data and taxonomic assignments*

127 Across the three Antarctic islands, we obtained a total of 1,181,155 raw sequences from
128 RNA, to combine with the 932,024 sequences previously described from DNA samples. After
129 quality filtering, ITS extraction and removal of chimeric, non-fungal or singleton sequences,
130 1,114,807 and 853,646 sequences remained in the RNA and DNA datasets, respectively.
131 There was variation in the number of sequences remaining per island, with the greatest
132 number of sequences being obtained for RNA in Bird Island soil (total of 386,029), and the
133 lowest for DNA in Signy Island soil (total of 226,472). The abundances of all taxa varied
134 across islands and between total and active communities (Figure 2). Overall, the
135 Leotiomycetes were the most frequently sequenced class, with members of the Helotiales
136 being the most commonly sequenced order. Members of the Eurotiomycetes,
137 Sordariomycetes and Microbotryomycetes were also frequent in soil (Figure 2).

138

139 *Abundances of endemic, cosmopolitan and bipolar fungi in active and total communities*

140 Endemic and cosmopolitan fungi showed differential patterns of abundance between the
141 active and total communities (Figure 3). Fungal OTUs identified as being endemic to
142 Antarctica were significantly more likely to have higher abundance in the active fungal

communities (Figure 4, $P<0.001$, observed proportion = 0.667, expected 95% confidence set: 0.617 – 0.654). In contrast, OTUs of cosmopolitan fungi were significantly more likely to have higher abundance in the total community than would be expected by chance (Figure 4, $P<0.001$, observed proportion = 0.480, expected 95% confidence set: 0.304 – 0.432). Bipolar fungi tended towards having higher abundances in the total community, but this difference was not significant (Figure 4, $P=0.077$, observed proportion = 0.550, expected 95% confidence set = 0.150 – 0.600).

Richness, evenness and taxonomic distinctness of total and active soil fungal communities

There was a positive correlation between the richness of fungal OTUs found within the DNA and RNA pools in each soil sample (for 1,000 iterations of the rarefied datasets, 95% confidence set of $r=0.629$-0.669; a plot of one random iteration is presented in Figure S1). Across the three islands, significantly fewer fungal OTUs were identified in the RNA pool than in the DNA pool from the same sample (Table 1). Although the difference was marginally non-significant ($P=0.05$), species evenness of RNA was consistently higher than that of DNA at each of the individual islands (Table 1), and species distribution curves show lower abundances of the most dominant fungi in the RNA libraries (Figure 5). Most of the more abundant OTUs were present in both total and active communities. Overall, around

161 24% of OTUs in the un-rarefied dataset were only present in the total community, and 22%
162 were only present in the active community, although these tended to be rarer OTUs.
163 Taxonomic distinctness did not differ between the two libraries on any of the three islands
164 (Table 1).

165 Across both active and total communities, fungal richness decreased with soil depth,
166 from an average of 147 OTUs at 2 cm to 133 OTUs at 8 cm, but the relationship was not
167 significant (χ^2=2.278, P=0.131), and there was no effect of soil depth on the magnitude of
168 the difference in fungal richness between total and active communities (χ^2=0.334, P=0.564)
169 – *i.e.*, the difference in fungal richness between total and active communities did not change
170 with depth.

171

172 *Compositional differences between total and active soil fungal communities*

173 Adonis analyses were used to test for differences in the composition of soil fungal
174 communities characterised using either RNA or DNA. Significant differences were detected
175 between the composition of active and total soil fungal communities across all islands
176 (r^2=0.082, pseudo-F=23.9, P<0.001) and within each of the three islands (Bird Island:
177 r^2=0.131, pseudo-F=13.312, P<0.001, Signy Island: r^2=0.400, pseudo-F=58.631, P<0.001,
178 Léonie Island: r^2=0.149, pseudo-F=15.419, P<0.001), demonstrating that when communities

179 were sampled from the same location, those characterised using DNA were more similar to
180 other samples characterised using DNA than to those characterised using RNA, and *vice*
181 *versa*. A generalized linear mixed effect model also showed that the proportion of fungi
182 shared between active and total communities decreased across all islands with increasing
183 soil depth (χ^2=13.378, P<0.001). Tests within each island showed that this effect was
184 present at Signy Island (χ^2=25.774, P<0.001), but not at the other two islands (Bird Island: χ
185 ^{2}=0.876, P=0.349; Léonie Island: χ^2=0.262, P=0.609).
186 Two classes of fungi showed significantly different patterns of abundance between
187 the total and active communities across the three islands: OTUs belonging to the
188 Leotiomycetes and the Microbotryomycetes more often showed higher abundance in the
189 total community than in the active community (P<0.001).
190 Abundances of fungal genera are provided in Table S1. Eight OTUs in the
191 Trichocomaceae (members of *Penicillium*, *Paecilomyces* and *Aspergillus*) were consistently
192 recorded as cosmopolitan taxa (Table S1). By contrast, all but three of the 86 OTUs in the
193 Chytridiomycota were found to be endemic to Antarctica (Table S1). The same pattern was
194 observed for lichen-forming fungi, with all but one of the 44 OTUs in the Verrucariales and
195 the Lecanoromycetes being recorded as endemic to the Antarctic (Table S1).
196

Discussion

The data reported here indicate that endemic Antarctic fungal taxa exhibited higher abundances in the active than in the total community of Antarctic soils, and, *vice versa*, that cosmopolitan soil fungi were more likely to occur in the total than in the active community. These observed patterns, which match those predicted by our conceptual framework (Figure 1), show for the first time that the distribution patterns of microbes can influence their abundances in active and total communities of geographically isolated soils. It is important to note that we have tested only for patterns of abundance consistent with our conceptual framework, and not for the mechanisms responsible for these observed patterns: for example, no quantification of spores, soredia, necromass or other metabolically less active tissues in soils was carried out in this study. A possible explanation for this observation is that endemic taxa are better adapted to the extreme environmental conditions encountered in the soils of the region, although what these specific adaptations are remains unknown. This view is supported by previous research showing that individual isolates of fungi, bacteria and flagellates from Antarctic soils and freshwaters have lower temperature optima for growth than those from tropical and temperate zones (Franzmann, 1996; Boenigk *et al.*, 2006; Tojo and Newsham, 2012). However, studies showing that endemic microbes are more active than cosmopolitan taxa in the natural environment through analyses of the

relative abundances of RNA and DNA have not, to our knowledge, previously been published in the literature.

Another possible explanation for the observations reported here is that cosmopolitan fungi have a tendency to produce large numbers of spores (which generally have low metabolic activity) in order to achieve long-range dispersal (Figure 1), which is consistent with the extremely large latitudinal ranges observed for non-endemic Antarctic soil fungal taxa (Cox *et al.*, 2016). Information on spore formation by fungi in soil, as well as the geographical limits of spore dispersal by specific taxa, is presently very limited. Some fungi with wind-dispersed spores have been shown to exist as distinct phylospecies on separate land-masses, indicating geographic barriers to spore dispersal (Shen *et al.*, 2002; Geml *et al.*, 2008). However, some fungi are suspected to be globally widespread (Pringle *et al.*, 2005; Jacquemyn *et al.*, 2017), and studies suggest that fungal spores have the potential to travel huge distances, including between South America and maritime Antarctica (Ingold, 1971; Marshall, 1996a; Brown and Hovmøller, 2002). In agreement with our conceptual framework, the data here show that the eight OTUs in the Trichocomaceae (members of *Penicillium*, *Paecilomyces* and *Aspergillus*), which on artificial media produce copious conidia typically measuring <6 μm × <4 μm (Domsch *et al.*, 1980), were recorded as cosmopolitan taxa in Antarctic soils. By contrast, in a pattern similar to that demonstrated in

233 plants, in which endemic taxa with limited ranges are often characterised by a lower
234 production of seed than is typical for species with wider distributions (Murray *et al.*, 2002;
235 Lavergne *et al.*, 2004), all but three of the 86 members of the Chytridiomycota, flagellated
236 fungi that typically disperse via zoospores in water films over distances of a few centimetres
237 (Powell and Letcher, 2014), were found to be endemic to Antarctica. Strikingly, the same
238 pattern was found for lichen-forming fungi, with all but one of the 44 OTUs in the
239 Verrucariales and the Lecanoromycetes being recorded as endemic Antarctic taxa,
240 suggesting that these fungi are inefficient at long-range dispersal. This observation is
241 consistent with previous reports of endemism in the Antarctic lichen flora (Lee *et al.*, 2008;
242 Jones *et al.*, 2015) and the finding that the majority of lichen propagules trapped from
243 Antarctica air are not spores, but soredia, measuring 30–60 μm (Marshall, 1996b).
244 Across all three islands, we consistently found that the richness of the active fungal
245 community was lower than that of the total community, in contrast with previous studies that
246 have found no differences between these two communities (Baldrian *et al.*, 2012;
247 Romanowicz *et al.*, 2016; Žifčáková *et al.*, 2016). This disparity may be driven by increased
248 preservation of relic DNA in the cold soils of Antarctica (Willerslev *et al.*, 2004), leading to
249 inflated species counts (Carini *et al.*, 2016), or because the relatively low overall richness of
250 soil fungi in this region (Cox *et al.*, 2016) results in fewer fungi being available for detection.

251 Despite lower richness at the sample level, a substantial proportion (>20%) of the fungal
252 taxa identified in the RNA sequencing library were not present in the DNA sequencing
253 library. This may indicate the presence of fungi which, although rare in the community in
254 terms of biomass, may be metabolically very active and functionally significant contributors
255 to ecosystem processes (Deacon *et al.*, 2006; Baldrian *et al.*, 2012). The active community
256 also showed higher levels of evenness, primarily caused by a reduction in the dominance of
257 the most abundant fungi. The most extreme example of this occurred on Signy Island, where
258 the most abundant OTU, an unidentified member of the Helotiales, represented 40% of the
259 DNA, but just 2% of the RNA, sequences in soil. This reduction in the abundance of
260 dominant fungal OTUs may also partly explain the large number of fungi only detected in the
261 RNA libraries, with the increased available sequencing depth enabling the detection of
262 additional rare active OTUs in the RNA pool.

263 The composition of total and active communities sampled from the same location
264 were found to significantly differ across and within each island, although the correlation
265 coefficient value for the across-island test was low, indicating that total compositional
266 differences may be small. We found little difference in taxonomic distinctness between the
267 total and active fungal communities, suggesting that the same taxonomic groups were
268 detected in both nucleic acid libraries, even if the abundances of some groups were very

269 different. However, as a relatively high proportion of these Antarctic fungi could not be
270 identified to even a low taxonomic resolution, we were limited in our ability to test for
271 taxonomic distinctiveness between active and total communities. Two classes, the
272 Microbotryomycetes and the Leotiomycetes, were found in higher abundances in the total
273 compared with the active community. The higher abundance of the Microbotryomycetes in
274 the DNA-derived community suggests that the frequent occurrence of Basidiomycetous
275 yeasts such as *Rhodotorula* spp. in Antarctic soils (Adams *et al.*, 2006; Newsham *et al.*,
276 2016) may not be reflected in their influence on soil functions such as nutrient cycling in the
277 natural environment. The finding that the Leotiomycetes, the most species rich and abundant
278 fungal class in the soils studied here, are frequent in the total community corroborates
279 previous research showing that members of this taxon are widespread in DNA-based
280 libraries constructed from Antarctic soils (Newsham *et al.*, 2016). Members of the Helotiales,
281 one of the most speciose orders of the class, are also frequent in the roots of *Deschampsia*
282 *antarctica* and *Colobanthus quitensis* (Upson *et al.*, 2009), the two plant species from under
283 which soil was sampled in the present study.

284 Total and active fungal communities both showed a significant shift in composition
285 across soil depths, in agreement with several other studies (Dickie *et al.*, 2002; Lindahl *et al.*,
286 2007; Talbot *et al.*, 2014). While some of these studies have highlighted how these

287 differences are often largely driven by shifts between mycorrhizal and decomposer fungi
288 across soil horizons, we found strong changes despite an absence of mycorrhiza-forming
289 fungi in Antarctic soils (Newsham *et al.*, 2009), suggesting shifts in composition relating to
290 changes in abundance of other root-associated fungal taxa such as the Helotiales (Upson *et*
291 *al.*, 2009), and/or because of changing carbon resources across soil depths. This is in
292 agreement with other studies that have shown depth-related structuring within fungal guilds
293 (Taylor and Bruns, 1999; Taylor *et al.*, 2014). We also anticipated that we might detect
294 consistent differences in the extent to which total and active fungal communities differed
295 across soil sampling depths (c.f. Herbold *et al.*, 2014), possibly because of deposition of the
296 spores of cosmopolitan fungi onto soil surfaces (Edman *et al.*, 2004). However, whilst there
297 was a significant reduction in the proportion of fungal OTUs shared between the two nucleic
298 acid pools at greater soil sampling depths, this pattern was only evident on one of the three
299 islands. This finding may therefore be dependent upon local ecosystem processes such as
300 spore deposition rates, or the extent of mixing between soil horizons.

301 Although the DNA and RNA used for characterising total and active soil fungal
302 communities in this study were simultaneously co-extracted and sequenced using the same
303 methodology, it is appropriate to issue caveats regarding the results of their comparisons.
304 Using sequence read abundance as a proxy for fungal abundance in the natural

305 environment may be inaccurate owing to variation between different taxa in ITS copy
306 numbers, as well as PCR biases arising from variations in the lengths of ITS regions
307 between species (Lindahl *et al.*, 2007). Although care was taken to match the denoising and
308 clustering algorithms used for constructing the DNA, RNA and worldwide reference libraries,
309 sequence processing steps such as denoising and OTU clustering can introduce errors or
310 sources of bias. The 97% cut-off used to group OTUs, although widely used in many
311 mycological studies (e.g. Baldrian *et al.*, 2012; Peay *et al.*, 2012; Talbot *et al.*, 2014), is
312 arbitrary and may fail to differentiate taxa with more conserved ITS regions. In addition, the
313 RNA and DNA extracted from soil were sequenced here on separate plates, which may
314 introduce stochastic differences between the abundance or presence of particular OTUs.
315 There remain gaps in our knowledge regarding how differential stability of DNA and RNA
316 molecules, and how the reverse transcription required for RNA processing, affect community
317 descriptions. However, although all of these caveats should be considered, they would only
318 affect the central question of this study – i.e. whether or not endemic and cosmopolitan
319 fungal taxa exhibit differential abundances in total and active communities – if biases are
320 concentrated in particular taxonomic groups in a way that correlates with differences in
321 endemism and cosmopolitanism.
322

Conclusions

Here, we found evidence for the first time that the traits related to fungal distribution patterns can affect their relative abundances within total and active communities of Antarctic soils. These patterns are consistent with a framework in which endemic and cosmopolitan fungi show differences in dispersal strategy, and degree of adaptation to the extreme environment of Antarctic soils, but further investigations are needed to identify the causes of such differences between total and active community profiles. Our analyses also indicated consistent differences in the community composition of fungi in the active and total communities of Antarctic soils, with the increased sequencing depth provided by RNA sequencing seeming to enable the detection of additional fungi. The findings suggest that studies based purely on the total microbial community, assessed by DNA-based analyses, may be biased towards specific taxa in a predictable manner.

335 **Experimental procedures**

336 *Sample collection*

337 Between October and November 2011, soil samples were collected from Bird Island

338 (54.0089° S, 38.0662° W), Signy Island (60.7107° S, 45.5849° W) and Léonie Island

339 (67.5984° S, 68.3561° W) in the sub-Antarctic, low maritime and high maritime Antarctic,

340 respectively. In order to achieve consistency between islands, soil was collected from under

341 populations of co-occurring *Deschampsia antarctica* Desv. and *Colobanthus quitensis*

342 (Kunth) Bartl., the only two native vascular plant species that occur in Antarctica. On each

343 island, 50 mL sterile centrifuge tubes (Corning Inc, Corning, NY, USA) were used to collect

344 soil samples by hammering them directly into the vertical walls of three pits at three depths

345 (2, 4 and 8 cm). Thus, a total of 27 soil samples were collected from across the three

346 islands. The three pits were a maximum of 1 km apart on each island, with an average

347 distance of 311 m separating them. The soil, kept on ice after collection and frozen at -80 °C

348 within 5 h, was freeze-dried to preserve fungal nucleotides.

349

350 *Nucleic acid extraction and amplification*

351 Total DNA and RNA were extracted simultaneously from five individual 50 mg samples,

352 taken from each of the tubes of homogenized soil (representing a total of 27 × 5 = 135

353 samples), using RNA PowerSoil Total RNA Isolation and DNA Elution Accessory kits (MoBio
354 Laboratories, Carlsbad, CA, USA). Extracted DNA was amplified in triplicate PCR reactions
355 using the primers ITS1F and ITS4 as described by Cox et al. (2016), with conditions
356 matching those described below for cDNA. Extracted RNA was treated with a Turbo DNA-
357 free kit (Life technologies, Carlsbad, CA, USA), checked for the absence of DNA using PCR,
358 and reverse transcribed using AccuScript High-Fidelity Reverse Transcriptase (Agilent,
359 Santa Clara, CA, USA) and random nonamers. The resulting cDNA was amplified in
360 triplicate PCR reactions using ITS1F (Gardes and Bruns, 1993) and ITS4 (White *et al.*, 1990)
361 primers. The ITS4 primer was modified with the Roche 454 A adapter and a 10-bp barcode
362 specific to each sample, allowing identification of different samples once pooled, and the
363 ITS1F primer was modified with the 454 B adaptor.
364 The triplicate PCR products were pooled and subsequently purified using AMPure
365 XP bead purification (Beckman Coulter, Inc, Brea, CA, USA) and quantified using a Qubit
366 dsDNA HS Assay (Life Technologies, Carlsbad, CA, USA) before normalization to consistent
367 concentration. The purified and normalized PCR products were run on a single plate, on the
368 454 Roche Titanium FLX platform at the Liverpool Centre for Genomic Research, at the
369 same time and under identical conditions to the DNA library.
370

371 *Sequence analyses*

372 The resulting RNA sequences were pooled with the DNA sequences (Cox *et al.*, 2016) and
373 processed together using the QIIME pipeline (Caporaso *et al.*, 2010). Sequences were
374 filtered to remove reads that were of low quality, less than 300 bp or greater than 1200 bp,
375 and were split according to barcodes. The remaining sequences were denoised to reduce
376 the influence of characteristic errors associated with 454 pyrosequencing, using the denoiser
377 algorithm available in QIIME (Reeder and Knight, 2010), and checked for potential chimeras
378 using UCHIME (Edgar *et al.*, 2011). Abundant sequences flagged as potential chimeras
379 through either denovo or reference based searches were manually checked, and confirmed
380 chimeric sequences filtered from the dataset. As the ITS1 region was not always fully
381 sequenced, the ITS2 regions of the remaining high quality non chimeric sequences were
382 extracted using ITSx (Bengtsson-Palme *et al.*, 2013) to remove flanking conserved regions
383 that can interfere with downstream sequence clustering. The ITS2 sequences were grouped
384 into Operational Taxonomic Units (OTUs) at 97% sequence similarity using USEARCH 6.1
385 (Edgar, 2010), approximating to species-level groupings. OTUs represented by a single
386 sequence were subsequently removed from the analyses, since they may often represent
387 erroneous sequences. Taxonomy was assigned to OTUs in QIIME, by running the BLAST
388 algorithm against the UNITE fungal database, uploaded on 20th November 2016.

389 Sequences were BLAST-searched (97%) against a previously described global
390 database of 32,376 soil fungal ITS2 sequences (Cox *et al.*, 2016). Briefly, this database was
391 compiled from 14 studies focused on fungi occurring in soils at 394 sites around the world,
392 with a minimum sequencing depth of 1,000 sequences per site. The ITS2 region of
393 sequences from all studies was extracted and clustered at 97% similarity using USEARCH
394 6.1 (Edgar, 2010), as implemented in QIIME (see Cox *et al.*, 2016 for more details). These
395 search results were used to assign each fungal OTU as either being endemic to Antarctica,
396 or cosmopolitan if it was found at sites elsewhere. An OTU was classified as bipolar if it
397 occurred north of the Arctic circle (66.56° N), but did not occur at latitudes in between. The
398 taxonomic assignments of the endemic, cosmopolitan and bipolar fungi are shown in Table
399 S1.
400
401 *Statistical analyses*
402 The five replicates from each soil tube were pooled and measures of fungal richness,
403 evenness, taxonomic diversity and taxonomic distinctness (Clarke and Warwick, 2001) were
404 calculated for each DNA/RNA pair. Linear mixed effects models were used to compare these
405 metrics between pairs of total and active samples, with island and sampling pit included as

random effects. A linear mixed effects model was also used to test whether the magnitude of differences in fungal richness between active and total communities changed with soil depth.

The R function Adonis (permutational MANOVA) (Oksanen *et al.*, 2017), was used to compare samples at the same depth within each pit, testing whether active community replicates were more similar to each other than they were to total community replicates, and *vice versa*. A generalized linear mixed effects model was used to test whether the proportion of fungi shared between paired samples of total and active communities changed with soil depth.

Permutational paired sign tests (using a custom VB script) were used to investigate whether endemic, cosmopolitan and bipolar fungi differed between total and active communities. This test was designed to identify differential abundances greater than the expected stochastic differences between sequencing runs. Based on BLAST searches against the global database of soil fungal ITS2 sequences (Cox *et al.*, 2016) each Antarctic OTU was labelled as endemic, cosmopolitan or bipolar. The test statistic was calculated as the proportion of OTUs in each of these three collective groups with higher overall abundance in the relevant community (either total or active, since this was a two-tailed test). This test statistic ensures that each OTU is weighted equally, and identifies consistent differences in abundance between communities. Alternative statistics based on differences

424 in sequence reads or OTU richness of each community were considered to give undue
425 weight to very abundant fungi with large numbers of reads, or rare fungi, which are
426 disproportionately more likely to appear in one library but not the other, respectively.
427 Significance was tested by comparing observed test statistics to a distribution of 1,000 null
428 values, generated by randomising labels (endemic/cosmopolitan/bipolar) across all OTUs.
429 As we were assigning cosmopolitan status based on matches to a database of fungi
430 detected using DNA-based analyses, it is possible that sequences detected using only RNA
431 were more likely to be assigned endemic status. Therefore, the results presented are for
432 tests carried out on only fungi that were detected in both nucleic acid libraries; however, the
433 results were qualitatively similar if all fungi were included in the test. The same tests were
434 also used to investigate whether OTUs in different taxonomic classes differed in abundance
435 between the two communities.
436 We used several approaches to ensure that differences in sequencing depth did not
437 affect the above analyses. For comparisons of metrics based on species richness,
438 communities were rarefied to the lowest common sequencing depth. The process of
439 rarefaction has recently attracted criticism in the context of comparisons of community
440 composition, due to the inherent loss of data involved (McMurdie and Holmes, 2014). In the
441 context of this study, rarefying can be considered conservative, as the loss of data reduces

442 the power to detect differences in abundance between DNA and RNA libraries (McMurdie
443 and Holmes, 2014). All tests were carried out on 1,000 iterations of the rarefied community
444 matrix, and also repeated on the unrarefied dataset. For comparisons of community
445 composition, Bray-Curtis distances were calculated from unrarefied, proportional
446 transformed data (Legendre and Gallagher, 2001), and repeated on rarefied data. All
447 conclusions were robust regardless of whether or not the data was rarefied – for
448 comparisons of species richness, evenness and taxonomic distinctness that are dependent
449 on equal sampling we present results from rarefied datasets; while for comparisons of
450 community composition, we present results from unrarefied, normalized data.

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TABLE 1 Differences in diversity indices between total (DNA-derived) and active (RNA-derived) soil fungal communities. Comparisons were made between 1,000 iterations of rarefied community matrices – the 95% confidence range of the mean is shown where variation is greater than 3dp.

		Bird Island†			Signy Island†			Léonie Island†			All islands‡		
		mean	χ^2	*P* value	mean	χ^2	*P* value	mean	χ^2	*P* value	mean	χ^2	*P* value
Richness	DNA	191.44 - 191.67	11.43	<0.001	146.67 - 148.00	11.57	<0.001	120.78 - 122.00	0.17	0.677	153.15 - 153.74	15.08	<0.001
	RNA	153.89 - 156.00			116.11 - 119.00			116.67 - 119.22			129.41 - 130.85		
Evenness	DNA	0.47	3.30	0.069	0.51	0.90	0.344	0.41	2.25	0.133	0.46	3.84	0.050
	RNA	0.51			0.53 - 0.54			0.48			0.51		
Taxonomic distinctness	DNA	76.98	2.34	0.126	77.09 - 77.13	3.62	0.057	70.82 - 70.88	0.26	0.609	74.97 - 74.99	0.52	0.470
	RNA	75.42 - 75.47			75.64 - 75.72			71.91 - 71.98			74.34 - 74.37		

Tests were carried out between 9† and 27‡ pairs of samples

625 **Figure Legends**

626 **Fig. 1.** Conceptual framework in which habitat specialisation and dispersal of fungal
627 taxa might predict their abundance in either the active or total communities of
628 Antarctic soils. See main text for further explanation.

629 **Fig. 2.** Heatmap showing the relative proportion of sequences belonging to different
630 taxonomic classes, sub-phyla or phyla in the total and active soil fungal communities
631 at the three Antarctic islands. Darker shades indicate higher relative proportions of
632 taxonomic groups.

633 **Fig. 3.** The numbers of RNA and DNA reads of each OTU, in each sample in which it
634 was recorded, as a function of each other. Note that OTUs endemic to Antarctica are
635 more abundant in the active fungal community (*i.e.*, occur below the 1:1 line) and that
636 OTUs which are cosmopolitan are more abundant in the total fungal community (*i.e.*,
637 occur above the 1:1 line). To allow valid comparisons of species abundance while
638 controlling for the total number of reads, the total number of reads per sample was
639 rarefied to the lowest common sequencing depth.

640 **Fig. 4.** Results of the permutation paired sign test. Filled circles and white bars show
641 observed values and 95% expected ranges, respectively. There was a lower than
642 expected proportion of endemic taxa with higher abundance in the total community,

643 while the proportion of cosmopolitan taxa with higher abundance in the total

644 community was greater than expected.

645 **Fig. 5.** K-dominance plots of total and active soil fungi communities at the three

646 Antarctic islands. The cumulative proportion of the overall community made up by the

647 10 most abundant OTUs on each island are shown, to illustrate the lower abundance

648 of the dominant fungi in the active communities.

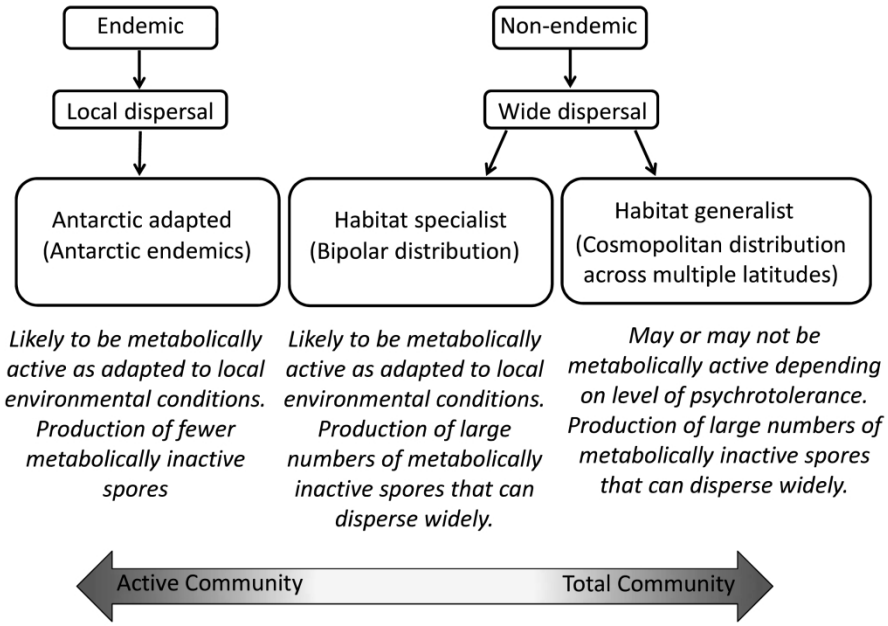
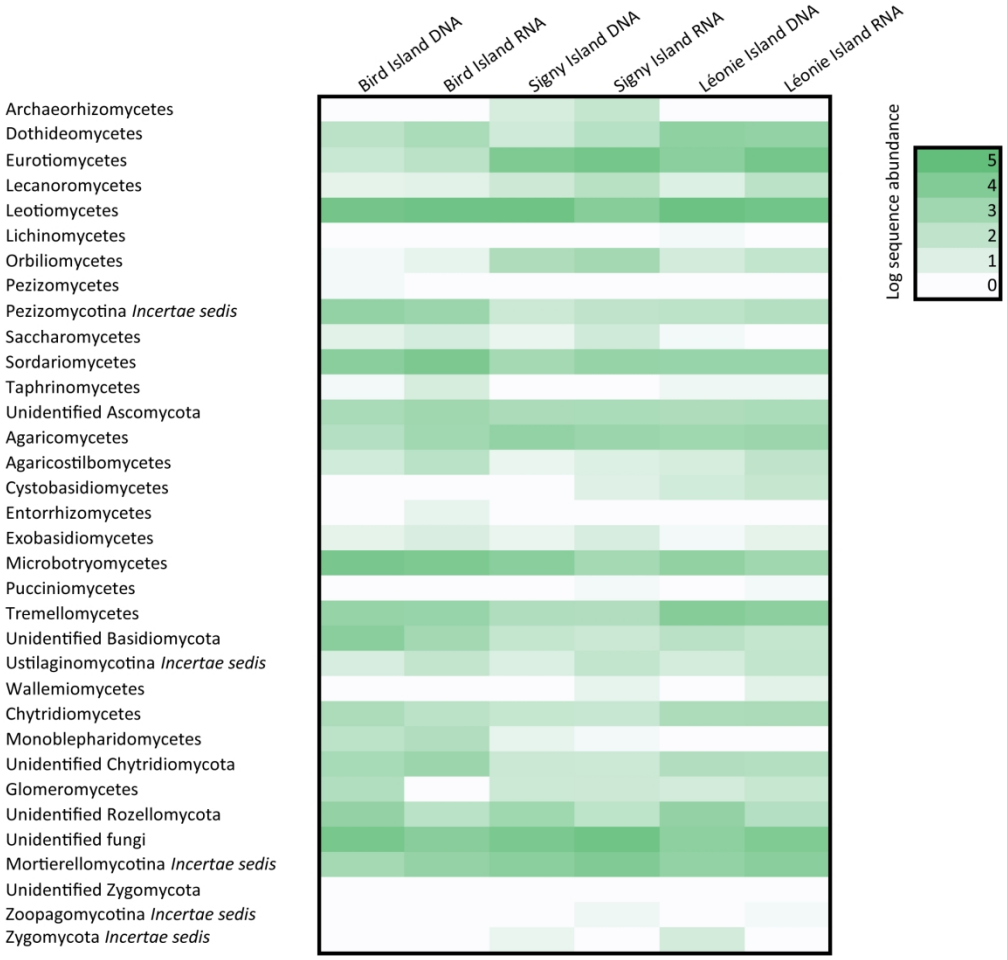


Fig. 1. Theoretical framework in which habitat specialisation and dispersal of fungal taxa might predict their abundance in either the active or total communities of Antarctic soils. See main text for further explanation.



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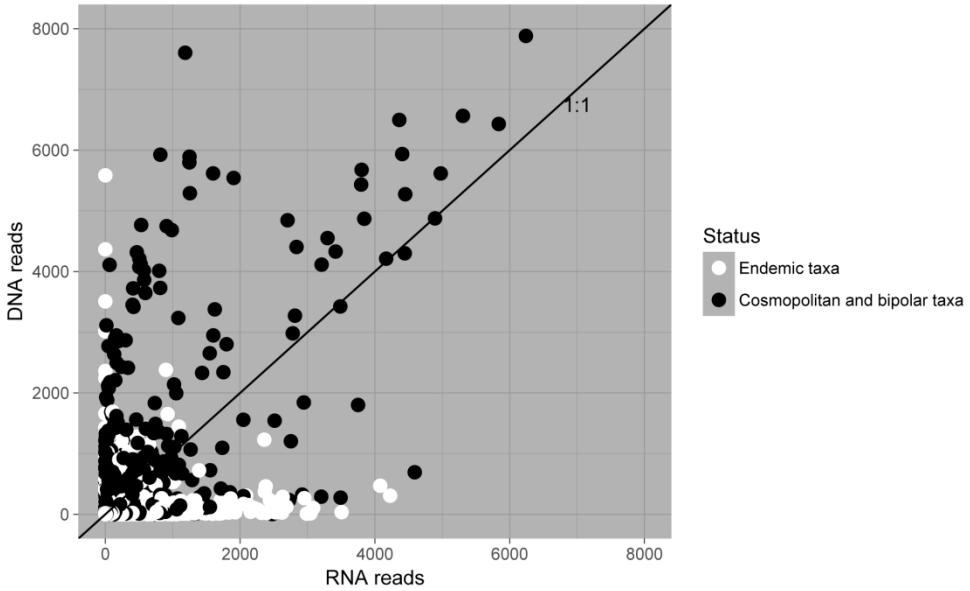

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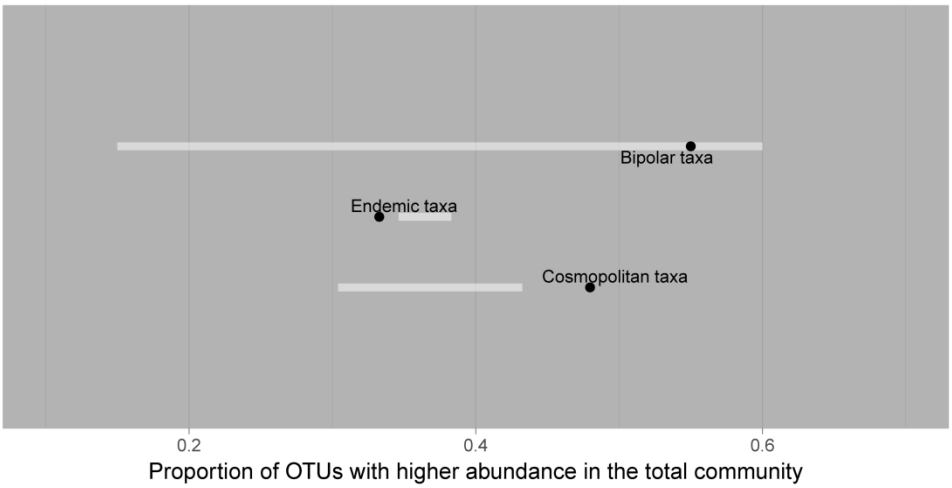

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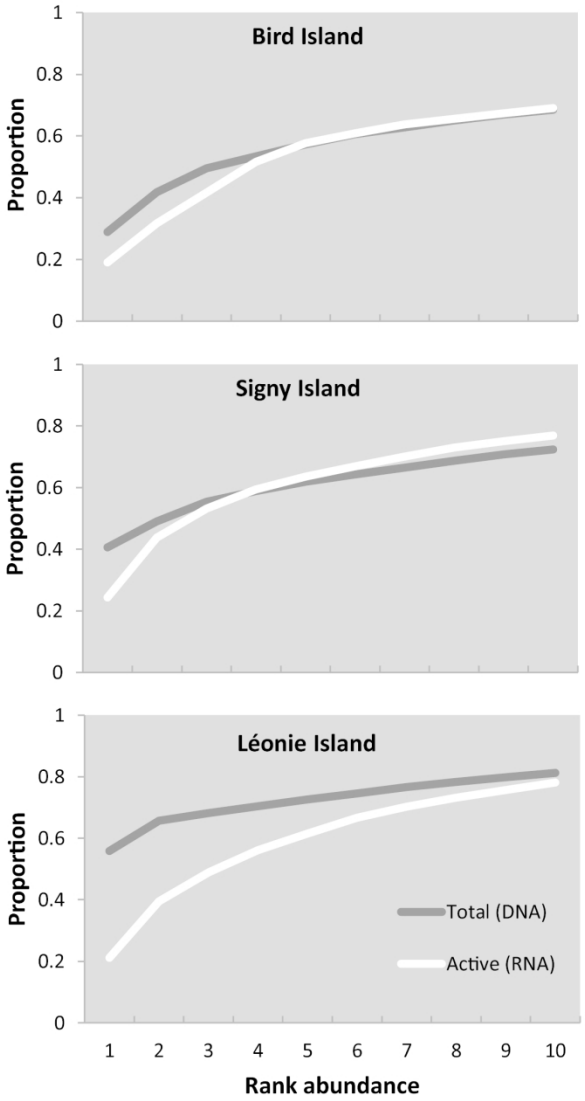

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