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Fungal colonization of exotic substrates in Antarctica

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Abstract Throughout the history of polar exploration and up to recent times, wood and other exotic materials have been brought to the Antarctic continent and left there. While the possible transportation of exotic fungal species on these materials is sometimes considered, the effects of these exotic substrates on indigenous fungal communities have not been previously evaluated. This study reports results from seven plots where organic materials were used in baiting studies to determine the fungal diversity present in soils. Four plots were on islands in the Palmer Archipelago on the Antarctic Peninsula and three at Ross Island, Antarctica. Samples of sterile wood and cellulose with and without nutrients added were buried in soil and left for either two or four years before being removed and evaluated for fungal colonization. There was a significant increase in fungal colony-forming units (CFU) from soil in direct contact with introduced, sterile wood and cellulose substrates compared to background soil levels. The type of substrate, 2 or 4 year incubation period in the field, or nutrient addition did not have a significant effect on culturable densities in soil. Fungal abundance on soil adhering to substrates was found to be similar to that found in non-polar soils indicating that lack of organic material may be the most significant limiting factor affecting densities of Antarctic fungal populations. Based on a high degree of colonization, these exotic substrates appear to have a significant effect on indigenous soil fungal abundance.

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Introduction

The early explorers of Antarctica left a remarkable historic legacy both in a cultural context and in a physical form with wooden structures and thousands of artifacts left on the continent. These materials have provided potential vectors for exotic species of fungi to be introduced into the continent. In addition, these exotic substrates represent a potential source of nutrients for use by indigenous soil fungi. Although woody plants do not currently grow on the Antarctic continent, there is strong evidence that Antarctic fungi are able to colonize and degrade introduced wood and other organic materials (Arenz and Blanchette 2009; Blanchette et al. 2004, 2010). These studies have documented the diversity of fungi at historic sites on Ross Island and the Peninsula region of Antarctica and their ability to attack wood. Some of these research sites are associated with the "Heroic Era" of Polar Exploration from 1901–1914 including those on Ross Island left by the expeditions of Robert Scott and Ernest Shackleton. Blanchette et al. (2004) first reported an unusual form of soft rot decay affecting wood of the buildings and artifacts caused by *Cadophora* species. This type of decay has subsequently been found to be prevalent in historic woods at many Antarctic sites (Arenz and Blanchette 2009; Blanchette et al. 2010; Held et al. 2005). Although the extremely cold and dry Antarctic environment strongly limits the rate of fungal degradation, investigations have shown that fungi can grow and cause significant impact over time. Held et al. (2005) found that during the Antarctic summer, environmental conditions conducive to fungal growth, including

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temperatures above 0°C and relative humidity above 75%, occurred for many weeks per year inside the historic huts on Ross Island.

The diversity of fungi at these sites and in soils from the immediate vicinity of the huts have been reported previously (Arenz et al. 2006) and included a variety of filamentous fungi and yeasts and a single zygomycete species. A similar study was undertaken on a large number of historic structures along the Antarctic Peninsula (Arenz and Blanchette 2009) and a similar mycological profile to the Ross Island sites with a high frequency of *Cadophora*, *Geomyces* and *Cryptococcus* species found. More recently, Blanchette et al. (2010) have reported that large numbers of degradative fungi have colonized the exterior wood of Shackleton's Cape Royds hut and this site has relatively high fungal diversity, presumably due to large carbon and nutrient input from the historic materials as well as introductions from the penguin colony nearby.

Since it is not known how introduced carbon and nutrient substrates influence Antarctic microbes, this study was done to determine the effect of exotic substrate introduction on the composition and abundance of fungal communities in the soil. Experimental field plots were established and sterile substrates buried at Ross Island in the Ross Sea Region and on the opposite side of Antarctica at the Palmer Archipelago. Substrates were chosen that were similar to building materials used in the construction of the historic huts as well as cotton as a source of cellulose without lignin. Effects on soil fungal communities were determined by taking colony counts based on dilution plating of soils adhering to the substrates upon removal, and comparisons were made from soils not in contact with substrates. Significant differences in colony counts between the two soil types would be indicative of substrate colonization/utilization. Utilization of wood as a baiting substrate has been used in the study of wood-inhabiting fungi in other environments such as mangrove swamps (Alias and Jones 2000; estuaries (Shearer 1972), beach sands (Tokoru 1984), freshwater streams (Latmore and Goos 1978) and Antarctic sea water (Pugh and Jones 1986). This study represents the first report of a wood baiting technique in an Antarctic terrestrial environment.

Materials and methods

Three biodiversity sampling plots were established on Ross Island in January of 2004. Four plots were also initiated near Palmer Station in March, 2005. The Ross Island plots were laid out approximately 100–200 m away from the locations of the historic structures at Hut Point, Cape Royds, and Cape Evans. The plots near Palmer Station on the Palmer Archipelago were installed in the "backyard" area of the Station on Anvers Island, on Humble Island, and at the Old Palmer Station site. In addition, a plot was placed on Limitrophe Island near Palmer Station in March, 2007 and a replacement plot was placed on the Old Palmer site since the original one established in 2005 had samples that were exposed at the soil surface due to the erosion of the top soil. In all cases, site locations for plots were chosen because they represented a relatively flat 2×6 m homogenous area of open soil free of large rocks.

Bait traps consisted of 1.5 cm×0.5 cm×10 cm pieces of three different types of wood, spruce (*Picea glauca*), southern yellow pine (*Pinus sp*.) and birch (*Betula papyrifera*) as well as small pieces of cellulose (unbleached cotton) placed in synthetic mesh bags. These four substrates were configured in an alternating arrangement in eight rows of 12 samples for a total of 96 baits per plot. Half of the substrates were vacuum saturated with a malt extract solution as a nutrient additive prior to placement in the field and the other half were vacuum saturated with distilled water as a control. All samples were sterilized via autoclaving and kept in sterile bags during transport to field sites. Samples were buried at the plot locations to a depth of 5 cm. Each row was connected via nylon string to a central aluminum pole in the plot to prevent loss of samples.

At intervals of 2 and 4 years, samples were removed from the Ross Island plots and taken for analysis. At the completion of each interval, half of the samples were removed (2006 and 2008) from Ross Island sites. In March, 2007, the 2 year samples from the Palmer Station site and the Humble Island site were removed for analysis. In March, 2009, the 4 year samples from the Palmer Station site and the 2 year samples from the Old Palmer and Limitrophe Island site were removed as they had been placed in 2007 as previously mentioned. The 4 year Humble Island site samples were unsuitable for analysis due to soil erosion which had exposed the substrates. Four soil samples were also taken from each plot at each corner at a depth of 5–10 cm to evaluate background soil microbial and edaphic characteristics.

Samples were removed from the soils using sterile scoops and sterilized gloves to prevent sample contamination. Individual samples were immediately placed into sterile Whirl-pack bags (Nasco, Fort Atkinson, WI) and kept at 4°C until arrival at the laboratory where they were kept at −20°C until processing.

Substrate samples were analyzed for fungal colonization by aseptically scraping off soil that adhered to the substrates and collecting it for culturing using a dilution series. One gram of scraped soil was added to an initial dilution of 9 ml sterile distilled H_20 and shaken to disperse soil particles. Further dilutions were made from this one in ten to a maximum of one in 100,000. One ml of each

dilution series was then added to the top of 100 mm plates of 1% Malt Extract Agar and Basidiomycete Select Agar (1.5% malt extract, 1.5% agar, 0.2% yeast extract, 0.006% benlate, and with 0.2% lactic acid and 0.001% streptomycin added after autoclaving) (Worrall 1999). Plates were incubated at both 20°C and 4°C for one week and observed for fungal growth. Plates were selected that had <200 colony forming units (CFU) for quantification. Morphologically distinct CFUs were quantified and subcultured at the margin of hyphal or yeast growth to obtain pure cultures.

Subcultures were grown for another week and then the hyphal or yeast surface was scraped for DNA extraction using an adapted chloroform extraction method. A small amount of fungal material (0.5 gm) was combined with 500 μl of lysis buffer (10% Tris pH 8, 10% 0.5M EDTA, 1% SDS) and vortexed with glass beads in a microcentrifuge tube. After removing the resulting solution to a clean tube, 275 μl 7 M ammonium acetate were added and the tube was incubated at 65°C for 5 min followed by 5 min on ice. Five hundred microliters of chloroform was added and the solution was vortexed for 1 min followed by 5 min centrifuge at 15,000 rpm. The aqueous fraction was removed to new tube and 1 ml isopropanol added, followed by 5 min incubation at room temp. The DNA was pelleted by 7 min centrifugation, after which the isopropanol was removed and the pellet washed twice with 70% ethanol. After the ethanol was removed, the pellet was allowed to dry for 30 min and re-suspended with 100 μl 1x TAE.

Following extraction, the internal transcribed spacer (ITS) regions 1 and 2 and the 5.8S gene of ribosomal DNA was targeted for PCR amplification and sequencing via the procedures of Arenz and Blanchette (2009). Sequences were compared to accessions in the GenBank database via BLASTn searching to find the most likely taxonomic designation.

Soil moisture was determined by weighing 5–10 gm soil before and after 2 h of oven-drying at 120°C followed by cooling overnight in a desiccation chamber. Preparation of a 1:2 soil/distilled H_2O solution was made before determining soil pH with an Accumet Research AR15 pH meter (Fisher Scientific, Pittsburgh, PA). Soil conductivity was based on a 1:5 soil/distilled H_2O solution measured with an Orion model 122 conductivity meter (Orion Research Incorporated, Boston, MA). Carbon and nitrogen levels of soil were determined using a Costech ECS 4010 CHNSO analyzer (Costech Analytical, Valencia, CA).

All statistical analyses were performed using the program R, version 2.9.2 (R Development Core Team 2009). The data were analyzed according to a split plot design with nutrient:year being treated as a whole plot treatment and substrate as a sub-plot treatment. Fungal colony-forming units (CFUs) were log-transformed and used as a response variable for a likelihood ratio test. Substrate type (spruce, birch, pine, cotton), nutrient (malt enrichment vs. sterile water), and year (2 vs. 4 year samples) were all used as predictor variables. In addition, the interaction effect of nutrient:year and all four treatment effects were tested jointly.

The effect of location was analyzed with a Dunnett-Tukey-Kramer (DTK) Pairwise Multiple Comparison Test to evaluate the significance of differences in fungal densities among the seven locations. Means of log-transformed CFU counts from soil collected in direct contact with the substrates were compared to "background" soils collected in the immediate area of the plots but not in direct contact with the substrates via a paired *T*-test.

Results

A total of 488 substrates were collected and analyzed for fungal CFU's and fungal diversity. In addition, 28 background soil samples (four from each location) were taken for analysis. Fungal abundance varied geographically (Fig. 1). There was a high incidence of sterile substrate-soil samples at the Ross Island plot sites at Cape Royds and Cape Evans where 91% of Cape Evans and 77% of Cape Royds samples produced no fungal colonies on the media used, compared to zero percent from all other sites. The high incidence of 0 CFUs from these two locations gave the overall data a non-normal distribution. For the purposes of the likelihood ratio test, the data from Cape Royds and Cape Evans were excluded.

Ross Island plots had lower soil moisture, percent carbon and nitrogen and higher conductivity (salinity) and pH (Table 1), compared to plots from the Peninsula. Average soil moisture of background Ross Island soils was 1.4% compared to 42.7% at Palmer Archipelago plots. Average percent carbon and nitrogen was 0.068% and 0.01%, respectively at Ross Island plots compared to 16.2% and 1.4% at Palmer Archipelago plots. Average conductivity and pH was 2701 μS/cm and 8.3, respectively at Ross Island plots and 363 μS/cm and 4.98 at Palmer Archipelago plots.

Palmer Archipelago sites had consistently high fungal counts, however, the location with the highest average fungal count from soils adhering to substrates was Hut Point on Ross Island (Fig. 1). Location had a significant effect on fungal count (ANOVA; $P<0.001$). Differences between substrates types within locations were not significant, but significantly higher fungal counts were observed in substrate-associated soil than background soils at the Hut Point, Old Palmer, Palmer Station, and Limitrophe locations (Fig. 1). Differences between fungal counts of substrate-associated soils and background soils at the Cape Royds, Cape Evans, and Humble Island locations were not

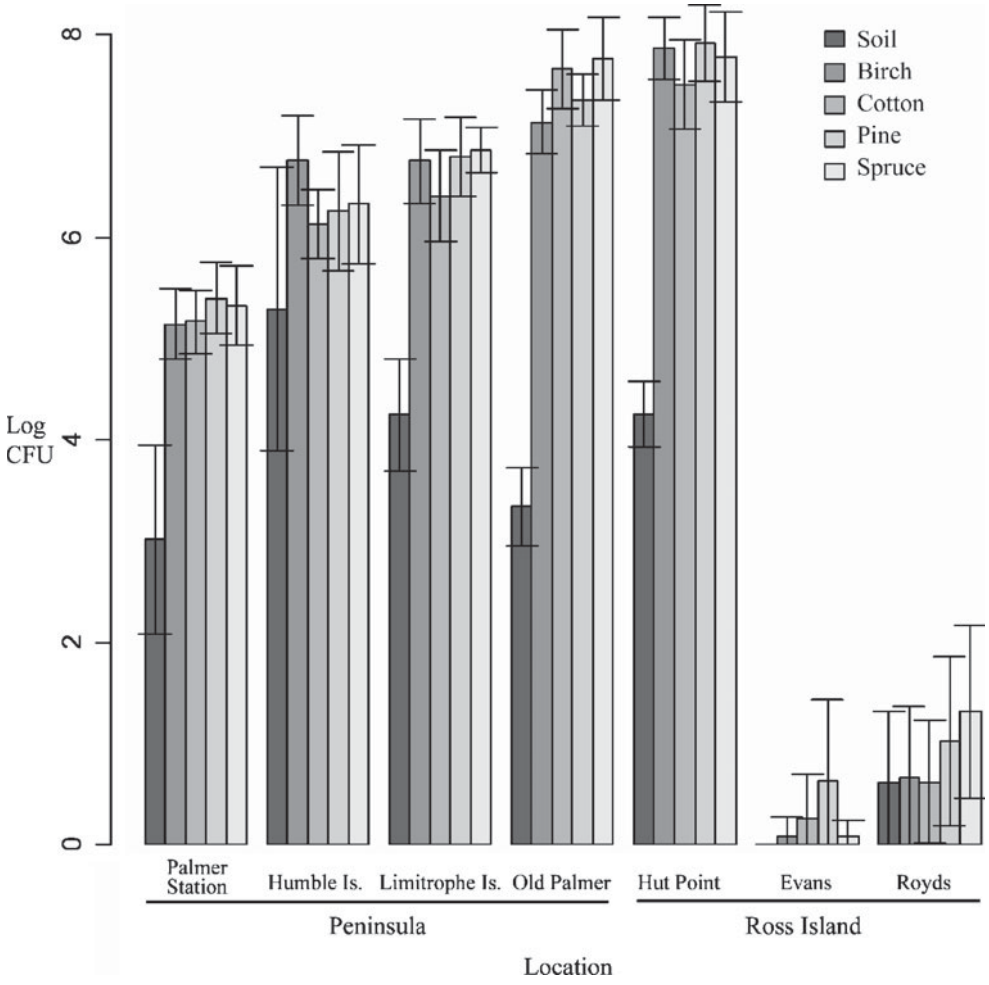

Fig. 1 Log fungal Colony Forming Unit (CFU) counts from soils associated with various substrates and location of study plot in Antarctica along with 95% confidence intervals

significant. Over all locations, there were significant higher logCFU values from the substrate contacted soils compared to logCFU of background soil collected from the plots (paired *t*-test; $P=0.013$).

There was no significant effect of nutrient additions on fungal counts compared to control substrates (Fig. 2) (likelihood ratio test; $P=0.12$). There was also no significant difference between CFU counts on 2 and 4 year samples in the locations where 4 year samples were obtained: Palmer Station, Hut Point, Cape Evans, and Cape Royds (likelihood ratio test; $P=1$). No significant differences were found by substrate type (likelihood ratio test; $P=0.17$) or by year, nutrient, substrate, nutrient:year interaction effect (likelihood ratio test; $P=0.29$).

Based on sequencing of the ITS region and BLASTn comparisons with GenBank, a total of 77 distinct opera-

Table 1 General soil characteristics at each of the seven experimental plot sites utilized in the fungal biodiversity studies with three sites on Ross Island and four sites on the Palmer Archipelago

	%Soil moisture	pH	EC (μS)	%N	%C	Fungal CFU per/g	Latitude	Longitude
Ross Island locations								
Hut Point	1.9	8.52	1635	0.013	0.119	22566	77° 50′S	166° 38′E
Cape Evans	1.4	8.05	2240	0.005	0.030	0	77° 38′S	166° 24′E
Cape Royds	0.9	8.23	4230	0.010	0.056	8	77° 33′S	166° 10′E
Palmer Archipelago locations								
Palmer Station	13.4	5.30	13	0.032	0.207	3259	64° 47′S	64° 04′W
Humble	73.1	5.03	1142	2.894	28.402	6235402	64° 46′S	64° 06′W
Old Palmer	11.1	6.06	35	0.018	0.288	2987	64° 46′S	64° 05′W
Limitrophe	73.4	4.39	264	2.719	35.748	29585	64° 48′S	64° 01′W

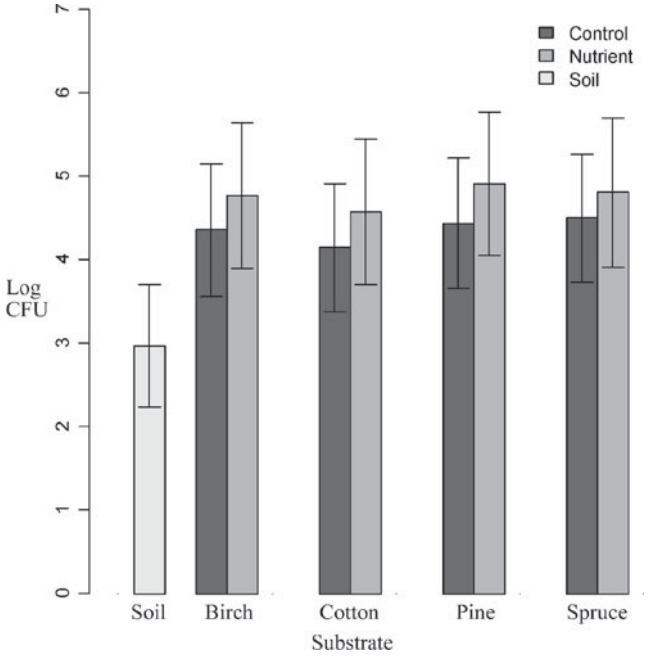

Fig. 2 Log fungal Colony Forming Unit (CFU) counts of soil associated with substrates by type and nutrient addition (control vs. nutrient) and background soil (soil) with 95% confidence interval

tional taxonomic units (OTUs) were identified in this study based on their unique sequences (Table 2). Twenty-four OTUs were found exclusively at Ross Sea sampling sites, 48 at Peninsula sites, and only five OTUs were found at both sites. Determined by total fungal counts, the phylum breakdown of isolated fungi was 94.3% ascomycetes and 5.6% basidiomycetes for the Ross Island sites and 99.2% ascomycetes, 0.7% basidiomycetes and 0.15% zygomycetes for the Peninsula sites. Using a criteria of 95% ITS sequence identity for confident genus placement, 59 of the taxa were found to be resolvable into 16 genera based on best Blast matches. The remaining 18 taxa either did not have a 95% match with any GenBank accession or those accessions were not identified to genus and taxonomic placement was not made. The most abundant genus based on total CFU count was *Geomyces* for both the Ross Island sites (78.6%) and the Palmer Archipelago sites (81%).

Although decomposition of substrates was not measured, soft rot decay was observed when selected wood samples were examined microscopically (data not shown). No evidence of any other type of wood decay was observed in any of the substrates. Substrate effect on soil communities was inferred through measurement of fungal CFU counts on soils adhering to the substrates when removed from the soil. However, if CFU counts were significantly higher than background soil CFU counts then it was inferred that fungal colonization of the substrates was occurring thus indicating substrate utilization. Based on these criteria, it can be concluded that fungal colonization of substrates was statistically significant at all locations apart from Cape Evans and Cape Royds on Ross Island and Humble Island on the Palmer Archipelago.

Discussion

A surprising finding from these results is the very large difference in fungal CFU counts between the Ross Island sites of Hut Point and those of Cape Evans and Cape Royds (Fig. 1). Measured fungal abundance on substrates was actually higher at Hut Point than at any other location in the study including the average of the Palmer Archipelago sites. One possible factor which could be responsible for this difference is the close proximity (<500 m) of the Hut Point site to McMurdo Station, the largest base in Antarctica currently occupied. Possible inputs to this site from McMurdo Station include windblown debris, foot traffic, and hydrocarbon contamination. An early study by Boyd and Boyd (1963) on bacterial abundance of Ross Island reported their highest count to be on Hut Point (9.8×10^8 CFU per gram soil), although the exact location of their sampling was not specified.

Other studies investigating human influences on microbial populations in Antarctica have reported similar effects. Line (1988) observed an increased abundance of microbes on "soil contaminated by man and animals" near the Australian Mawson Base. Kerry (1990) did not quantify fungal abundance but noted a difference in fungal taxa between Mawson Base and outlying areas "which may be attributable to human activity" though she did note a decrease in *Geomyces pannorum* (the most abundant genus in our study) frequency in petroleum contaminated soils.

G. pannorum, previously known as *Chrysosporium pannorum*, is one of the most frequently reported fungal species from Antarctica, and since it is found in so many places it has been suggested to be indigenous (Vishniac 1996). This cosmopolitan fungus has also been isolated from a range of sites worldwide but seems to be more frequently found compared to other fungi in cold environments, including the Arctic (Bergero et al. 1999; Ozerskaya et al. 2008). Previous studies have shown *Geomyces* to be a leaf litter decomposer and also to increase in abundance at lower temperatures (Ivarson 1974). This fungus has also been found to be keratonophilic and associated with feathers in soils (Marshall 1998). Gene flow by avian vectors from temperate locations where wood is abundant could be responsible for the ability of Antarctic strains to utilize exotic woody substrates. Measurement of cellulase activity has been reported from *G. pannorum* isolated from Ross Island historic huts at both Cape Evans (Duncan et al. 2006) and Hut Point (Duncan et al. 2008) as well as *Cadophora*, *Penicillium* and *Cladosporium* spp. Although

Table 2 Taxa isolated with comparisons to the best BLASTn match with the NCBI GenBank database. Total number of isolations is indicated as well as percent of overall fungal CFU counts compared to Ross Island and Antarctic Peninsula totals

Best Blast Match	ID %	Overlap	Total	Ross Is. %	Peninsula %	Accession number
Ascomycete						
Antarctomyces psychrotrophicus [AM489755]	100	502/502	1		0.001	HM589308
Ascomycete sp. BC15 [DQ317348]	99.8	499/500	3	0.004		HM589309
Ascomycota sp. Cq_ByP_D8 [FM207645]	100	458/458	3		1.755	HM589310
Cadophora malorum strain CCF3784 [FJ430743]	100	518/518	47	15.458		HM589311
Cadophora sp. 5R24-1 [DQ317330]	98.6	493/500	1		0.006	HM589312
Cladosporium cladosporioides [FM998717]	100	500/500	1	0.043		HM589313
Cladosporium sp. SGSGf38 [EU715666]	100	468/468	3	<0.001	0.012	HM589314
Curvularia inaequalis [FM163616]	99.8	523/524	1	0.009		HM589315
Debaryomyces hansenii strain MA09-AK [GQ458025]	100	587/587	1	<0.001		HM589316
Debaryomyces sp. CBS 5572 [AM992909]	99.8	549/550	2		0.001	HM589317
Debaryomyces sp. CBS 5572 [AM992909]	100	597/597	28		0.040	HM589318
Ericoid mycorrhizal fungal sp. shylhd15 [EU888618]	97.7	474/485	15		1.057	HM589319
Ericoid mycorrhizal fungal sp. shylhd15 [EU888618]	97.8	485/496	5		0.200	HM589320
Ericoid mycorrhizal fungal sp. shylhd15 [EU888618]	99	494/499	3		0.562	HM589321
Fungal endophyte isolate PS1 [EU167914]	99.8	514/515	1	0.193		HM589322
Fungal endophyte sp. ECD-2008 isolate 109 [EU686037]	93.6	443/473	2		0.025	HM589323
Fungal endophyte sp. P503B [EU977278]	100	502/502	1		0.107	HM589324
Fungal sp. AB21 [FJ235954]	99.8	535/536	1	<0.001		HM589325
Fungal sp. AB47 [FJ235980]	100	527/527	8		0.341	HM589326
Fungal sp. AB48 [FJ235981]	99.6	506/508	4		0.475	HM589327
Fungal sp. AB53 [FJ235986]	100	507/507	1	0.006		HM589328
Fungal sp. UFMGCB 2692 [FJ911879]	90.9	390/429	1	1.017		HM589329
Geomyces pannorum strain ASIGP1 [DQ779788]	93.3	477/511	1		<0.001	HM589330
Geomyces pannorum strain ASIGP1 [DQ779788]	92.9	443/477	1		0.076	HM589331
Geomyces pannorum strain VKM FW-857 [DQ189229]	99.6	497/499	37	6.037		HM589332
Geomyces pannorum strain VKM FW-857 [DQ189229]	99.8	450/451	2	0.063	0.009	HM589333
Geomyces sp. BC7 [DQ317337]	100	465/465	126	59.576	63.170	HM589334
Geomyces sp. BC7 [DQ317337]	98.9	441/446	6		1.954	HM589335
Geomyces sp. BC7 [DQ317337]	99.6	491/493	1		0.043	HM589336
Geomyces sp. BC7 [DQ317337]	100	556/556	2		0.699	HM589337
Geomyces sp. BC9 [DQ317339]	99.8	445/446	2	10.026		HM589338
Geomyces sp. BC9 [DQ317339]	100	483/483	10	0.658		HM589339
Geomyces sp. FFI 30 [AJ608960]	99.8	515/516	43		5.205	HM589340
Geomyces sp. FFI 30 [AJ608960]	99.4	475/478	1		<0.001	HM589341
Geomyces sp. FMCC-3 [DQ499473]	100	467/467	3		0.043	HM589342
Geomyces sp. FMCC-3 [DQ499473]	98.6	501/508	7		0.683	HM589343
Geomyces sp. T489/9b [AY345348]	99.8	510/511	9	1.192	9.219	HM589344
Holwaya mucida [DQ257357]	96.7	493/510	23		5.952	HM589345
Holwaya mucida [DQ257357]	96.7	491/508	2		<.001	HM589346
Iceman fungal clone T2709 [X88771]	98.6	490/497	6		0.627	HM589347
Onygenales sp. 7R38-1 [GU212403]	100	497/497	1	<0.001		HM589348
Penicillium swiecickii [AJ608946]	99.6	492/494	1		<0.001	HM589349
Phoma sp. 2 [AF218789]	100	565/565	1	<0.001		HM589350
Phoma sp. 2 [AF218789]	98.8	576/583	1	0.010		HM589351
Rhizoscyphus ericae isolate pkc29 [AY394907]	98.4	498/506	1		<0.001	HM589352
Stictis radiata isolate MW6493 [AY527309]	82.9	435/525	1		<0.001	HM589353
Thelebolaceae sp. BC18 [DQ317351]	100	511/511	3	0.011	<0.001	HM589354

Table 2 (continued)

Best Blast Match	ID %	Overlap	Total	Ross Is. %	Peninsula %	Accession number
Trichophyton eboreum [AJ876907]	93	465/500	1		0.001	HM589355
Uncultured ascomycete [AM901737]	99.5	557/560	4	0.006		HM589356
Uncultured Calycina clone IIP4-11 [EU516683]	98.7	476/482	1		0.002	HM589357
Uncultured fungus clone IVP1-32 [EU516819]	96	510/531	4		0.001	HM589358
Uncultured Pezizomycotina clone L10 [DQ273336]	97	506/521	84		6.890	HM589359
Basidiomycete						
Basidiomycota sp. 6/97-58 [AJ279465]	98.8	569/576	1		0.182	HM589360
Cerrena unicolor strain xsd08079 [FJ478121]	100	612/612	1		0.003	HM589361
Cryptococcus foliicola [AY557600]	100	478/478	6	0.004		HM589362
Cryptococcus sp. BC25 [DQ317361]	99.8	511/512	80	4.626		HM589363
Cryptococcus sp. YSAR10 [AM922286]	99	606/612	135		0.284	HM589364
Cryptococcus victoriae strain CBS 8884 [AF444645]	100	468/468	6	0.359		HM589365
Fungal sp. BB12 [FJ235998]	99.5	423/425	15	0.010		HM589366
Fungal sp. BB7 [AM901895]	100	540/540	2		0.220	HM589367
Rhodotorula laryngis strain CBS2221 [AF190014]	100	551/551	6	0.009		HM589368
Rhodotorula slooffiae strain PYCC [AF190014]	100	446/446	2	<0.001		HM589369
Rhodotorula sp. BC22 [FJ807685]	100	526/526	28	0.659		HM589370
Rhodotorula sp. NRRL Y-17502 (58)	100	534/534	3	0.023		HM589371
Stereum hirsutum strain dd08027 [AF444615]	96	571/595	1		0.003	HM589372
Uncultured basidiomycete [AM901895]	99.8	550/551	1		0.005	HM589373
Uncultured fungus clone S114 [FJ820602]	98.5	595/604	1		0.002	HM589374
Uncultured fungus clone S176 [FJ820664]	100	624/624	2		0.001	HM589375
Zygomycete						
Fungal sp. WD12a [EU240043]	99.1	567/572	2		0.014	HM589376
Mortierella sp. 04 M 158 [AY842393]	100	572/572	6		0.001	HM589377
Mucor hiemalis f. corticola strain CBS 106.09 [AY243950]	99.8	593/594	15		0.028	HM589378
Mucor hiemalis strain CBS 201.65 [DQ118992]	99.5	606/609	6		0.083	HM589379
Mucor hiemalis strain CBS 201.65 [DQ118992]	100	597/597	11		0.003	HM589380
Uncultured fungus clone 5 [AY702074]	97	583/600	1		0.002	HM589381
Uncultured fungus clone IIN5a12 [EF635686]	91.1	571/627	1		<0.001	HM589382
Uncultured fungus clone IIN5a12 [EF635686]	91.2	572/627	2		<0.001	HM589383
Uncultured soil fungus clone T1-A4-ITSFL [GU083146]	99.8	541/542	8		0.014	HM589384

the wood degradation potential of this genus has not been demonstrated, the results from this study suggest it can at least utilize wood as a nutrient substrate.

The next most common fungal genus at the Ross Island plots was *Cadophora* (15.5% of total CFU's). The genus *Cadophora* has been previously found to attack wood causing a soft rot type of decay in historic woods throughout ice-free regions of Antarctica (Arenz and Blanchette 2009; Arenz et al 2006; Blanchette et al. 2004, 2010; Held et al. 2005). The two taxa that appeared in the next greatest abundance in Palmer Archipelago plots after *Geomyces* and *Cadophora* (Table 2) were difficult to resolve taxonomically based on GenBank comparisons. This includes the third most common group (6.9%) that had a best Blast match to an "uncultured Pezizomycotina clone" with 97% identity. Similarly, the fourth most common group (6%) was an OTU which had a best Blast match with *Holwaya mucida* but only a 96% identity, and *Holwaya* is a genus that has not been previously reported from the Antarctica. Based on these ambiguities it is difficult to resolve the potential indigenous/exotic nature of these two groups of fungi. These fungi need additional phylogenic analyses to determine their appropriate taxonomic placement. The fifth most common genus was *Cryptococcus* (5%), a very frequently reported fungal genus from Antarctic soils (Bridge et al. 2009) including endemic species from the McMurdo Dry Valleys (Vishniac 1985; Vishniac and Hempfling 1979; Vishniac and Kurtzman 1992).

Prior to the early polar explorers, wood had not existed on the Antarctic continent in non-fossilized form since the

late Tertiary period (5–2 mya) and the transition from "magellenic subpolar forests to true tundra" (Poole and Cantrill 2006). Native fungal species would presumably not have high selection pressure to maintain specific enzymes for wood degradation but the presence of bryophytes and algae could provide pressure to maintain cellulose-degrading enzymes. It is possible that genes for wood-degrading enzymes were brought into this environment during recolonization events following periodic glacial retreats including after the last glacial maximum, 22–17 kya (Convey et al. 2009). Environments with low species richness are thought to favor generalist ecological strategies over specialization (Vazquez and Stevens 2004) and these results would suggest that the dominant fungal genera in these exotic substrates (*Geomyces* and *Cadophora*) have the capacity to utilize a wide range of possible nutrient sources.

As an overall average from the two major areas studied, the four sites on the Peninsula had higher fungal log CFU counts than the three sites on Ross Island (6.32 Peninsula, 3.14 Ross) as well as nearly twice the species richness based on OTUs reported (Table 2). Given that these locations were approximately 12.5° farther north in latitude, this is not surprising as a decrease in both biodiversity and species abundance has been noted at higher latitudes (Hillebrand 2004). There is also a substantial difference in climate between these two locations. While both are located at coastlines, the Palmer Archipelago sites experience warmer temperatures, with −3°C as the mean annual temperature compared to −17°C at Ross Island, as well as increased humidity and precipitation, 810 mm mean annual rain equivalent compared to 190 mm at Ross Island (NCDC 1996). Periodic rainfall at the Palmer sites also reduces soil salinity levels compared to Ross Island where rain rarely, if ever, occurs. Increased levels of vegetation are probably also an important factor in higher fungal abundance with the Palmer Archipelago sites of Humble Island and Limitrophe Island both having large quantities of mosses and even grasses (*Deschampsia antarctica*), although the sites of Old Palmer and Palmer Station are predominantly unvegetated. All three experimental sites on Ross Island were devoid of macroscopic vegetation and had soils which largely consisted of volcanic scoria.

As stated previously, substrates at Cape Evans and Cape Royds were frequently found to have no CFU presence based on the media used for isolation. This was surprising considering the abundance of fungal growth previously found in and around the Cape Evans and Cape Royds Huts, whereas Discovery Hut at Hut Point had relatively little fungal activity in the structure (Arenz et al. 2006; Blanchette et al. 2004, 2010). The reason for this is likely due to the different microclimates inside the structures at these locations, with Discovery Hut being much colder and dryer in general than the other two (Held et al. 2005) due to differences in hut design. Heterogeneity in the Antarctic soil environment may also be responsible for the different results. Fine-scale variability of water potential in Antarctic Dry Valley soils has been noted to have a significant impact on nematode activity (Treonis and Wall 2005) and extreme environments lacking vegetation in general are thought to be highly heterogeneous with regard to suitable habitats on a small spatial scale (Wall and Virginia 1999). It is possible that the plots chosen at Cape Evans and Cape Royds simply had a larger proportion of unsuitable fungal habitat. Hut Point background soils were also found to have higher moisture, percent carbon and nitrogen and lower conductivity then Cape Royds and Cape Evans background soils (Table 1). All of these factors were associated with greater fungal abundance based on a wide-ranging companion soil study (Arenz and Blanchette 2010).

Although there were significant differences between CFU counts on Palmer Archipelago plots, in general, there was much less variation among the Peninsula sites as compared to the Ross Island locations and no instance of soil lacking CFUs was found in any of the samples from the Peninsula. Greater incidences of vegetation contributing organic matter to the soil as well as more frequent precipitation events has likely accelerated soil development and led to less heterogeneity in terms of suitable habitat for soil microorganisms at the Peninsula sites.

Microbial life in Antarctica is subjected to a number of potential limiting factors. Extreme low temperatures (including frequent freeze-thaw cycles), reduced moisture, high salinity, high UV radiation, and low nutrient availability may vary significantly from location to location but are all potential challenges that microbes must overcome. To what extent each of these individual factors is limiting microbial populations has been difficult to ascertain as they are often not variables which can be directly separated and studied individually. These results indicate that relatively high levels of fungal CFUs can be reached by adding an organic substrate to the soil. The average fungal abundance based on CFU counts from substrate-contacted soil in the 10^7–10^8 range are comparable to those found in temperate ecosystems and much greater than the commonly found range of 10^4–10^5 CFU range in background soils reported in this and other Antarctic soil studies (Baublis et al. 1991; Connell et al. 2006; Gesheva 2009).

Although these research results suggest that the lack of carbon and nutrient substrates may be one of the more important limiting factors for fungi in Antarctica, it is surprising that the addition of nutrients to wood and cotton did not have a significant effect on fungal abundance (Fig. 2). It seems that the wood or cellulose substrate itself provided an adequate nutrient source for significant

increases of CFU formation and having an additional input of available nutrients is not overriding other limiting factors. Although moisture in these added substrates likely equilibrated to background soil moisture levels soon after being placed in the ground, the organic substrates may have also served as a moisture sink and represented a more consistent source of moisture for fungi during periodic freeze/melt/evaporation cycles. The most significant finding from these results is that the presence vs. absence of a "substrate" affected the fungal abundance from the soil more than any other measured variable.

It is likely that at least some of the fungi that have been detected from around these historic areas have been introduced through human actions. This could have been through original transportation of these substrates, subsequent human visitation to these sites, or general human activity in the area. However, their continued survival, growth potential, and fecundity in the relatively cold, dry, oligotrophic, and highly saline soils of Antarctica could be strongly constrained. Environmental selection pressure may exclude many of these exotic fungi over time in favor of native fungi that are highly adapted to the harsh soil conditions. The fungal genera with the highest abundance represented by substrate isolations in this study (*Geomyces* and *Cadophora*) appear to be indigenous to Antarctica based on their widespread reported occurrence throughout the continent and large diversity of species found there (Arenz and Blanchette 2009; Arenz et al. 2006; Blanchette et al. 2010; Bridge et al. 2009). These organisms also appear to have a very broad capacity to degrade a variety of substrates and a generalist ability to capture and utilize nutrients given their ability to quickly grow and proliferate on the exotic materials we introduced. This anthropogenic addition of carbon, nitrogen and other nutrients into the Antarctic environment appeared to have benefited indigenous fungal populations to a greater extent than introduced species that are likely restricted by polar environmental conditions. The very high increases in indigenous Antarctic fungal CFU counts on substrate-associated soils compared to background soils supports the hypothesis that these fungi are primarily restricted by nutrient limitations in their natural soil environments.

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