Fungi in Antarctica: a circumpolar study of biodiversity in soils and historic structures

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Dedication

This dissertation is dedicated to my beautiful wife, Kate and our two lovely children, Nigel and Phyllis.

Abstract

The results from investigations reported in this dissertation provide important new information on fungi found in Antarctica and their role in the degradation of historic wood as well as factors that influence their distribution and abundance. This dissertation has 4 chapters that report different investigations that were carried out in Antarctica. The first is a survey of fungal diversity found at historic sites on the Antarctic Peninsula. The second chapter details results from wood and cellulose fungal baiting traps on Ross Island and the Antarctic Peninsula. The third chapter is a wide ranging survey of fungal soil biodiversity found on Ross Island, the Antarctic Peninsula, and the Dry Valleys as well as evaluation of soil characteristics which are correlated with fungal abundance. The fourth chapter is a report on the current condition of East Base, the oldest standing US base in Antarctica and an important Polar heritage site.

Antarctica is the most remote and isolated continent on Earth and is generally thought to have low biodiversity due to environmental extremes. These relatively simple ecosystems are important to study because they can be used to improve understanding of more complex systems world-wide that are difficult to analyze directly. The isolation of the continent, extreme environmental conditions and the lack of functional redundancy in ecosystem processes make it particularly vulnerable to human disturbance and require improved understanding. The results of this research support the hypothesis that fungal abundance and distribution are generally linked to the presence of primary producers and their effect on carbon and nitrogen quantities in the soil. Experiments introducing sterile plant-derived nutrient sources (wood and cellulose) to soils resulted in increased fungal

abundance up to three to four orders of magnitude greater than background soil levels. This suggests that the extremes of the Antarctic environment (low moisture, high salinity, cold temperatures) are primarily affecting fungi by limiting the distribution of flora and direct effects on the fungi are relatively less important as these indigenous soil fungi appear well adapted to Antarctic environment.

A survey of fungal diversity near historic sites and areas where materials were introduced to the Antarctic Peninsula reveals a very similar composition to those affecting historic sites on Ross Island. The fungi found in greatest abundance were species of *Geomyces* and *Cadophora*. These two genera also formed a large percentage of the fungal colonization of buried nutrient substrates. The frequent reports of these fungi from many areas in Antarctica and the large diversity of species found indicates they are well adapted to their environment and suggests they are indigenous to Antarctica. The dominance of these fungi on human-introduced material indicates direct human influences may be of more benefit to generalist indigenous decomposer fungi which are pre-adapted to the environmental extremes rather than human-introduced fungi which may be better adapted to utilizing these substrates but not well adapted to the Antarctic environment. It also supports the hypothesis of indigenous Antarctic fungi being primarily limited by nutrient availability.

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Chapter 1

Investigations of fungal diversity in wooden structures and soils at historic sites on the Antarctic Peninsula

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Investigations of microbial diversity in Antarctica are important to begin to understand ecosystem functioning and decomposition processes. This study documents fungi at nine historic sites on the Antarctic Peninsula collected from wooden structures, other organic materials and soils during a joint National Science Foundation and British Antarctic Survey expedition in 2007. Many of these sites had wooden structures built by the British during the World War II Operation Tabarin, but others visited included the American "East Base" on Stonington Island and the Swedish hut on Snow Hill Island. Fungi were cultured on several different media, pure cultures obtained and identified by DNA sequencing of the internal transcribed spacer (ITS) region. *Cadophora* species previously found to attack historic wooden structures on Ross Island, Antarctica were found at all but one location sampled in the Peninsula region. Fungi causing decay in the historic wooden structures and artifacts and those causing mold problems inside the structures are of great concern and conservation efforts are urgently needed to help preserve these important polar heritage structures. Results presented also expand our knowledge on the identity of fungi present throughout the Antarctic Peninsula region and provide insights into the organisms responsible for decomposition and nutrient recycling.

Introduction

The Antarctic Peninsula has been the target of significant exploration over the past decades and many nations constructed huts or relatively large bases there in the early and middle part of the last century. The British in particular established a large presence in this region for not only scientific and geographic exploration but also to reinforce their claims of territorial sovereignty. The buildings and materials left behind from these expeditions still exist and many have been classified as historic monuments by the Antarctic treaty system but unfortunately deterioration and decay are causing significant problems for their long term preservation.

Past research on historic wooden structures in other regions of Antarctica has found that they are affected by both abiotic and biotic forms of degradation. Abiotic forms include wind erosion, oxidation of metals (Otineo-Alego et al. 2000) and salt defibration of wood fibers (Blanchette et al. 2002). Biotic deterioration is largely due to fungi which can cause both disfiguring mold growth on the surface of artifacts and a soft-rot type of wood decay (Blanchette et al. 2004*a*). The fungi found to be responsible for decaying wood were several species of *Cadophora* including *C. malorum*, *C. luteo-olivaceae* and *C. fastigiata* as well as several previously undescribed *Cadophora* species. Although this past research took place in the Ross Sea region of Antarctica where it is relatively very cold and dry, environmental monitoring inside the huts has revealed that during the Austral summers conditions that are conducive to fungal growth (temperatures above 0˚ C and relative humidity above 80%) occur frequently (Held et al. 2005). A survey of fungal diversity at these Ross Sea historic sites has found that the most commonly

isolated species affecting the historic wood and artifacts were also isolated from soils around the structures and from more distant and isolated areas (Arenz et al. 2006). A number of cosmopolitan species were also identified that had not been previously reported from Antarctica although in relatively low frequency.

Historic sites on the Antarctic Peninsula have not previously been the subject of mycological investigations and little is known about the microorganisms present in these areas. If these polar heritage sites are to be adequately preserved, it is important for conservators to know what factors are contributing to the deterioration and decay of the wood and artifacts. Since the Antarctic Peninsula has a relatively warmer and more humid climate than the Ross Sea region of Antarctica, degradation processes likely take place faster and a greater abundance of diverse fungi could be expected. This study was done to determine fungal diversity present at nine historic sites on the Antarctica Peninsula in woods, organic materials and soils, to make comparisons to other previous studies completed on the opposite side of Antarctica in the Ross Sea region, and to learn more about these organisms and their role in polar ecosystems.

Materials and Methods

Nine sites on the Antarctic Peninsula were visited in January 2007 from the British Antarctic Survey (BAS) ship HMS Endurance (Table 1.1). The sites consisted of seven historic British bases; Base A (Port Lockroy Station), Base D on Hope Bay, Base E on Stonington Island, Base F (Wordie House), Base V (View Point Station), Base W on Detaille Island and Base Y on Horseshoe Island (Fig. 1.1). In addition, Otto

Nordenskjöld's Swedish expedition hut on Snow Hill Island was visited as well as the oldest standing American base in Antarctica, East Base, on Stonington Island (Fig. 1.2). Small samples (<100 g) of wood, soil, and other organic artifacts such as textiles, rope, and foodstuffs were collected, placed in sterile bags and kept at 4 °C onboard the HMS Endurance until they were brought to the laboratory at the University of Minnesota for processing at the conclusion of the 4 week trip.

Fungi were isolated from the samples by culturing on several types of media including malt extract agar (1.5% Difco malt extract), a semi-selective medium to culture basidiomycetes (Worrall 1999) (1.5% Difco malt extract, 0.2% yeast extract, 0.006% benlate, with 0.2% lactic acid and 0.001% streptomycin sulfate added after autoclaving), Difco potato dextrose agar and Sabauroud dextrose agar (4% Dextrose, 1.5% agar, 1% polypeptone peptone, with 0.001% cycloheximide and 0.005% chloramphenicol added after autoclaving). Isolates were cultured at 8 and 20 °C. Pure cultures were obtained after sub-sampling.

DNA was extracted by a phenol-chloroform procedure. This procedure was modified from Zhong and Steffenson, 2001, in that mycelium and spores were collected by scraping the surface of the agar plate containing the pure isolate, rather than filtering of a liquid culture. Also, 700 µl of the lysis buffer was used instead of 7 ml. Ribosomal DNA was amplified via PCR with the primers ITS1 and ITS4 (Gardes and Bruns, 1993). PCR amplification was performed with Amplitaq Gold PCR Master-mix and 1 µl template DNA using manufacturer's instructions (Applied Biosystems, Foster City, CA). A MJ

Research PTC Minicycler (Watertown, MA) was used with the following profile: 94 °C for 5 min; 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min followed by a final extension step of 72 °C for 5 min. PCR products of appropriate size (500-600 bp) were verified by electrophoresis of the amplicons on a 1% agarose gel with a SYBR green 1 (Molecular Probes, Eugene, OR) pre-stain and transilluminating with a Dark Reader DR45 (Clare Chemical Research, Denver, CO). Sequencing of both strands was performed using ITS1 and ITS4 primers with the ABI PRISM Dye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems) and an ABI Prism 377 automated DNA sequencer. DNA sequence data were analyzed by Chromas software (Technelysium Ltd., Helensvale, Australia) and assembled into a consensus sequence. The sequences were compared to others in GenBank using BLASTn (Altschul et al., 1990) to find the best match.

Selected wood samples were cut with a cryostat freezing microtome and observed with a Hitachi S3500 scanning electron microscope.

Results and Discussion

A total of 186 samples were analyzed from all locations (Table 1.1), a large portion of which came from East Base since this was a very large historic site and the main focus of the deterioration assessments during the event. From these samples, 81 taxa were identified from 295 fungal isolations (Table 1.2). Ascomycetes (58) were dominant among the isolates with a smaller component of basidiomycetes (17) and zygomycetes (6). The predominant genus isolated was *Cadophora*, which represented 18% of all

isolates, and *Cadophora* spp. were found at all sites except Nordenskjold's hut on Snow Hill Island. Other frequently isolated genera were *Penicillium* (14%) *Geomyces* (11%), *Nectria* (5%), *Rhodotorula* (5%), *Cryptococcus* (4%), *Phoma* (3%) and *Hormonema* (3%). Of the 295 total isolations, 54 were obtained from soil samples. The most frequently isolated genera from these soil samples were *Geomyces* (24%), *Cryptococcus* (19%), *Rhodotorula* (13%), *Pseudeurotium* (7%) and *Nectria* (6%).

At the Phylum level, 82% of the isolates were ascomycetes, 15% basidiomycetes and 3% zygomycetes. When comparing this with previous work at Antarctic historic sites located in the Ross Sea region (Arenz et al. 2006), a similar result with 75% ascomycetes, 21% basidiomycetes and 1% zygomycetes representing the total isolates was found. This agrees with a recent review (Ludley and Robinsons, 2008) concerning "decomposer" basidiomycota in the Polar Regions that found few studies reporting filamentous basidiomycetes from Antarctic regions south of the sub-Antarctic. Most of the basidiomycetes found in maritime and continental Antarctica tend to be yeasts, and in the present study, basidiomycetous yeasts predominated. Arenz et al. (2006) recently found only yeasts and no filamentous basidiomycetes in an extensive study in the Ross Sea region. Although more research needs to be done, it is likely that ascomycetes function as the primary filamentous decomposers in the more southerly Antarctic soil ecosystems. It should be noted that filamentous fungi are less commonly isolated in general in the Dry Valleys and basidiomycete yeasts dominate those soil fungal communities (Atlas et al. 1978, Connell et al. 2008, Vishniac 1996). Dry valley soils are characterized by very arid

conditions and low nutrient availability, even by Antarctic standards, and this may exclude filamentous ascomycetes for the most part.

Additional comparisons of this study with the Ross Sea region study (Arenz et al. 2006), show a large amount of similarity in specific fungal taxa. As a percentage of all 82 taxa identified, 62% were from identical genera, 34% were also identical species and 21% also matched the same GenBank accessions. As a percentage of total number of isolates this increased to 77%, 64% and 46% for similar genera, species and GenBank accessions, respectively. Even though the Ross Sea region study area (Arenz et al. 2006) was 10 degrees further south and roughly 3,500 km away from the historic sites in this study, the overall profile of fungal taxa appeared to be very similar.

The most noticeable difference found was the large presence of *Penicillium* spp. in this study (14%) compared to the 2% observed in the Ross Sea area investigation (Arenz et al. 2006). Many of these species are part of a larger group of *Penicillium* spp. (subgenus Penicillium) which have commonly been associated with spoiling of refrigerated foods and as a dominant fungal component of subglacial Arctic ice (Sonjak et al. 2006). These species are relatively rare in soils in temperate areas (Frisvad and Samson, 2004) and were not found in soils analyzed in the study reported here from the Peninsula region, however, they have been reported from soils in some other previous studies carried out from the Peninsula area (Hughes et al. 2007) as well as the Ross Sea region (Göttlich et al. 2003, Sun et al. 1978) and even Dry Valley soils (Connell et al. 2006).

The large presence of *Geomyces* spp. (11%) is similar to what was previously observed at the Ross Island region (14%) (Arenz et al. 2006). *Geomyces* spp. have been observed frequently in Antarctic soils (Baublis et al. 1991, Tosi et al. 2005, Connell et al. 2006) and their dominance in soil samples in this study (24%) is not surprising. Marshall (1998) speculated that they may have a keratoniphilic role in ornithogenic soils. All sites in the present study were in coastal areas with some amount of bird life and Port Lockroy on Goudier Island is in the middle of a thriving Gentoo penguin rookery.

The most significant finding of this study is the high prevalence of *Cadophora* spp. among the historic sites visited (18% of all isolates). At least one isolate was found from samples at all nine sites except Nordenskjöld's hut on Snow Hill Island. Not only has this genus emerged as the primary group of decay fungi attacking wood and complicating conservation efforts at historic sites in the Ross Sea region (Blanchette et al. 2004*a*) but has also been isolated from historic wood from the Canadian High Arctic (Blanchette et al. 2008). Its high relative abundance on wood and artifacts in historic sites studied in this research is a cause for concern for conservators working to preserve these structures and artifacts in this environment. Results of scanning electron microscopy confirm that wood from where *Cadophora* species were isolated had an extensive soft rot type of decay (Fig. 1.3) and in laboratory studies this genus has been found to cause the same type of decay (Held et al. 2006). Soft rot decay has been found to be a dominant type of wood decay in extreme environments where conditions do not support white and brown rot fungi (Blanchette et al. 2004*b*).

Although collecting time at each location did not allow for an inspection of all woods, sufficient sampling was done to provide a preliminary account of the fungi present and the condition of the historic structures. The overall condition of the sites and artifacts ranged widely from those in very good condition such as Base Y on Horseshoe Island and Nordenskjöld's hut on Snow hill Island to those in much more deteriorated condition such as Station W on Detaille Island, Base D in Hope Bay and East Base on Stonington Island (Fig. 1.4). The condition of the buildings was usually affected by the state of the roofs, which at Base Y and Nordenskjöld's hut were largely intact, leading to relatively dry conditions inside the structures inhibiting fungal growth. However, the roofs at Station W and Base D had lost much of their water protecting properties and consequently a significant amount of moisture entered the huts and large fungal blooms were observed with significant damage to artifacts inside. A detailed assessment of deterioration and decay at East Base, has been published separately (Arenz and Blanchette, 2008).

This study demonstrates that a diverse range of fungi are present at these historic sites, many of which have a circumpolar distribution. Although a few filamentous basidiomycetes were found, which are rare in Antarctica, the predominant group of decay fungi appears to be ascomycetes in the *Cadophora* genus. This genus is present in abundance at all but one of the historic sites on the Antarctic Peninsula visited and preservation efforts need to consider these destructive microbes when trying to conserve these structures and artifacts. The environmental conditions on the Antarctic Peninsula appear more conducive to wood decay than the colder and drier Ross Sea region and

decay rates are likely to be much greater. The widespread and high relative abundance of *Cadophora* spp. suggests they are important organisms in soil ecosystem dynamics and nutrient recycling in these Polar Regions. This report also provides insight into the many other fungi that can be found in Antarctica, some of which undoubtedly play important roles for successful ecosystem functioning. Since little is known about these microorganisms there is a need for continued study to better understand how they survive in these harsh environments and what nutrients they are utilizing in the absence of exotic substrates left behind by expeditions.

Site	Location	National Affiliation	Year of construction	Coordinates	Samples
Base D	Hope Bay	British	1945	63°24'S, 56°59'W	12
Base V "View Point"	Duse Bay	British	1953	63°32'S, 57°23'W	10
Nordenskjöld's Hut *	Snow Hill Island	Swedish	1902	64º27'S, 57º12'W	19
Base A "Port Lockroy" *	Goudier Island	British	1944	64°49'S, 63°30'W	16
Base F "Wordie House" *	Winter Island	British	1947	65°15'S, 64°16'W	15
Base W	Detaille Island	British	1956	66°52'S, 66°48'W	18
Base Y *	Horseshoe Island	British	1955	67°49'S, 67°18'W	20
East Base *	Stonington Island	USA	1940	68°11'S, 67°00'W	59
Base E *	Stonington Island	British	1961	68°11'S, 67°00'W	17

Table 1.1. Sampling locations with original national affiliation of the site, year of construction and number of samples analyzed for fungi. * Designated as a historic monument by the Antarctic Treaty.

Best Blast Match	Percent	Overlap	Hope Bay	View Point	Snow Hill	Port Lockroy	Wordie House	Detaille Island	Horseshoe Island	East Base	Base E	Total	Sample	Accession
Ascomycetes														
Alternaria tenuissima strain bxq41209 [EF556213]	100	547/547			2							*2*	w	FJ235934
Ascomycete sp. 6/97-36 [AJ279469]	99.6	544/546		1								*1*	w	FJ235935
Ascomycete sp. BC12 [DQ317343]	99.6	529/531				3						*3*	w	FJ235936
Ascomycete sp. BC15 [DQ317348]	100	520/520	1	1					2	2	1	*7*	w,s,o	FJ235937
Ascomycete sp. WRCF-A1[AY618686]	99.8	458/459							1			*1*	w	FJ235938
Aureobasidium pullulans [AF121283]	99.2	523/527								1		*1*	w	FJ235939
Cadophora fastigiata [DQ317326]	100	546/546								7		*7*	w	FJ235940
Cadophora luteo-olivacea strain 18 [DQ404348]	99.8	594/595				1		2		5		*8*	w,o	FJ235941
Cadophora malorum [DQ317328]	100	559/559		5		9	2	3	1	10	6	*36*	w,o	FJ235942
Cadophora melinii strain 435 [DQ404351]	97.9	521/532	1									*1*	s	FJ235943
Candida novakii strain NRRL Y-27346 [DQ911449]	97.8	260/292							2			*2*	w	FJ235944
Candida zeylanoides [AB278160]	100	622/622								1		*1*	w	FJ235945
Cladosporium cf. subtilissimum CBS 172.52 [EF679390]	99.8	511/512			2							*2*	w,o	FJ235946
Cladosporium cladosporioides isolate 2728 [EU272532]	100	528/528			1				2	1		*4*	w	FJ235947
Coniochaeta ligniaria [AY198390]	99.8	550/551					2		2	2		*6*	w,o	FJ235948
Dactylaria sp. olrim414 [AY781221]	99.8	457/458					1					*1*	w	FJ235949
Dactylella tenuifusaria strain CBS617.95 [DQ494371]	92.5	136/147					1					*1*	w	FJ235950
Davidiella macrospora strain CBS 138.40 [EU167591]	100	538/538			2							*2*	w,o	FJ235951
Debaryomyces hansenii voucher MCCC2E00222 [EF194843]	100	613/613			2	1	1					*4*	w,s	FJ235952
Dothioraceae sp. BC10 [DQ317340]	100	562/562		1					1			*2*	w	FJ235953
Exophiala sp. BC36 [DQ317336]	100	565/565		2				3	1			*6*	w,o	FJ235954
Foliar endophyte of Picea glauca sp. Q1 [AY561213]	99.4	517/520					3			2	1	*6*	w,o	FJ235955
Fungal endophyte isolate 5 [EU747834]	99.6	511/513			1							*1*	o	FJ235956
Geomyces pannorum strain VKM FW-969 [DQ189225]	100	528/528	1						4	3		*8*	w,s	FJ235957

Geomyces sp. BC7 [DQ317337]	100	556/556			1	4			3	5	2	*15*	w,o,s	FJ235958
Geomyces sp. BC9 [DQ317339]	100	552/552				1		1		4		*6*	w,o,s	FJ235959
Geomyces sp. FMCC-3 [DQ499473]	99.5	547/550								1		*1*	w	FJ235960
Geomyces sp. T489/9b [AY345348]	99.6	516/518								1		*1*	s	FJ235961
Helotiales sp. MK9 [EU700254]	96.4	459/476			1							*1*	s	FJ235962
Hirsutella sp. ICMP14250 [EF029185]	97.4	526/540					1					*1*	w	FJ235963
Hormonema dematioides E99156 [AY253451]	100	574/574		2					1	6	1	*10*	w,o	FJ235964
Iceman fungal clone T2709 [X88771]	97.4	484/497	1							3		*4*	w,s	FJ235965
Nectria sp. olrim170 [QY805576]	99.6	472/474					1					*1*	w	FJ235966
Nectriaceae sp. BC4 [DQ317333]	98.2	514/517	1	1	1	2	1	2	1	4		*13*	w,o,s	FJ235967
Oidiodendron griseum strain UAMH 8528 [AF062796]	97.5	512/525								1		*1*	w	FJ235968
Penicillium camemberti isolate 944 [DQ681327]	99.8	530/531		1								*1*	w	FJ235969
Penicillium chrysogenum isolate NRRL 35688 [EF200101]	100	573/573			1							*1*	o	FJ235970
Penicillium commune isolate NRRL 35686 [EF200099]	100	562/562		1	1							*2*	w	FJ235971
Penicillium corylophilum strain FRR 802 [AY373906]	100	559/559									1	*1*	o	FJ235972
Penicillium mali [AF527056]	100	547/551			1		1	1		3		*6*	w,o	FJ235973
Penicillium roquefortii strain FRR 849 [AY373929]	100	570/570							1	1		*2*	w	FJ235974
Penicillium sp. BC37 [DQ317344]	100	571/572	4		3		1	3		11	3	*25*	w,o	FJ235975
Penicillium sp. Psf-2 [EF660439]	100	561/562			1							*1*	o	FJ235976
Penicillium verrucosum strain ATCC 44407 [AY373937]	100	562/562								1	2	*3*	w,o	FJ235977
Phialocephala dimorphospora isolate olrim310 [AY606304]	99.4	473/476					1					*1*	w	FJ235978
Phoma herbarum strain ATCC 26648 [AY293800]	98.5	525/533		1	3					2		*6*	w,o	FJ235979
Protoventuria alpina strain CBS 140.83 [EU035444]	99.6	552/559		2					1			*3*	w,o	FJ235980
Pseudeurotium bakeri strain MCJAxII [DQ529304]	99.2	514/517				2		1				*3*	s	FJ235981
Pseudeurotium desertorum CBS 986.72 [AY129288]	95.6	488/510		1								*1*	s	FJ235982
Rhinocladiella atrovirens strain WRCF-AB3 [AY618683]	99.2	508/512					2					*2*	w	FJ235983
Sydowia polyspora strain CUBC-F1 [DQ787428]	99.8	508/509									1	*1*	w	FJ235984
Thelebolaceae sp. BC17 [DQ317350]	100	524/524							1			*1*	s	FJ235985
Thelebolaceae sp. BC18 [DQ317351]	99.8	526/527				1					1	*2*	w	FJ235986

Ulocladium botrytis strain UAMH 7841 [AY625070]	99.8	576/577			2							*2*	w,o	FJ235987
Uncultured ascomycete [AM901709]	100	585/585									1	*1*	w	FJ235988
Uncultured ascomycete [AM901737]	99.7	572/574	2		2	2	1		1			*8*	w,s	FJ235989
Uncultured fungus isolate PS21 [EF159531]	99.6	501/503		1								*1*	w	FJ235990
Uncultured Pyronemataceae clone DGGE band BD6 [DQ317369]	99.7	338/339					1					*1*	w	FJ235991
Basidiomycetes														
Amyloathelia crassiuscula [DQ144610]	92.1	537/646					3					*3*	w	FJ235992
Antarctic yeast CBS 8913 [AY040666]	98.2	561/571									1	*1*	w	FJ235993
Cerinosterus luteoalbus strain WRCF-AW12 [AY618667]	95.4	413/433							1			*1*	w	FJ235994
Cryptococcus gastricus strain ATCC 32042 [EU266562]	99.8	607/608	2						1			*3*	s	FJ235995
Cryptococcus sp. BC25 [DQ317361]	99.8	491/492					1					*1*	o	FJ235996
Cryptococcus sp. NRRL Y-17490 [AF444449]	100	594/594								1		*1*	o	FJ235997
Cryptococcus sp. YSAR10 [AM922286]	99.8	587/588						1				*1*	s	FJ235998
Cryptococcus terricola ATCC:32040 [EU252550]	99	591/597		1								*1*	s	FJ235999
Cryptococcus victoriae strain CBS 8884 [AF444645]	100	512/512	1			1		1	3			*6*	s,w	FJ236000
Glacial ice basidiomycete GI254 [AF261656]	100	592/592			1						2	*3*	s,o	FJ236001
Rhodotorula laryngis strain CBS2221 [AF190014]	100	548/548	1						1			*2*	w,s	FJ236002
Rhodotorula minuta strain CBS 7296 [AF444620]	97.6	520/532								1		*1*	w	FJ236003
Rhodotorula sp. BC22 [DQ317357]	100	560/560				1		1			2	*4*	w,o,s	FJ236004
Rhodotorula sp. BC29 [DQ317365]	99.8	550/551	1	1				1	4			*7*	w,o,s	FJ236005
Sistotrema brinkmannii strain ATCC 26295 [DQ899094]	100	581/581	4				2	1				*7*	w,o	FJ236006
Sporidiobolus salmonicolor strain PYCC 5245 [EF592129]	99.8	572/573									1	*1*	w	FJ236007
Uncultured basidiomycete [AM901895]	99.8	584/585	1									*1*	s	FJ236008

Zygomycetes														
Helicostylum elegans [AB113014]	99.5	648/651								2		2	w,o	FJ236009
Mortierella sp. 04M 158 [AY842393]	100	627/627	1									1	s	FJ236010
Mortierella sp. Finse 23-07-02 [AJ541798]	97.7	618/631								3		3	w	FJ236011
Mortierella sp. WD35C [EU240119]	99.8	624/625							1			1	s	FJ236012
Mortierellaceae sp. BC21 [DQ317354]	99.8	614/615							1			1	s	FJ236013
Mucor hiemalis strain CBS 201.65 [DQ118992]	99.8	627/627	1									1	s	FJ236014
	Total		*23*	*22*	*28*	*28*	*26*	*21*	*37*	*84*	*26*	*295*		

Table 1.2. List of taxa isolated in this study including best blast match with percent identity and overall nucleotide overlap of the ITS region. Number of isolations by location and total isolations are also included as well as sample type; (w) wood, (o) other or (s) soil.

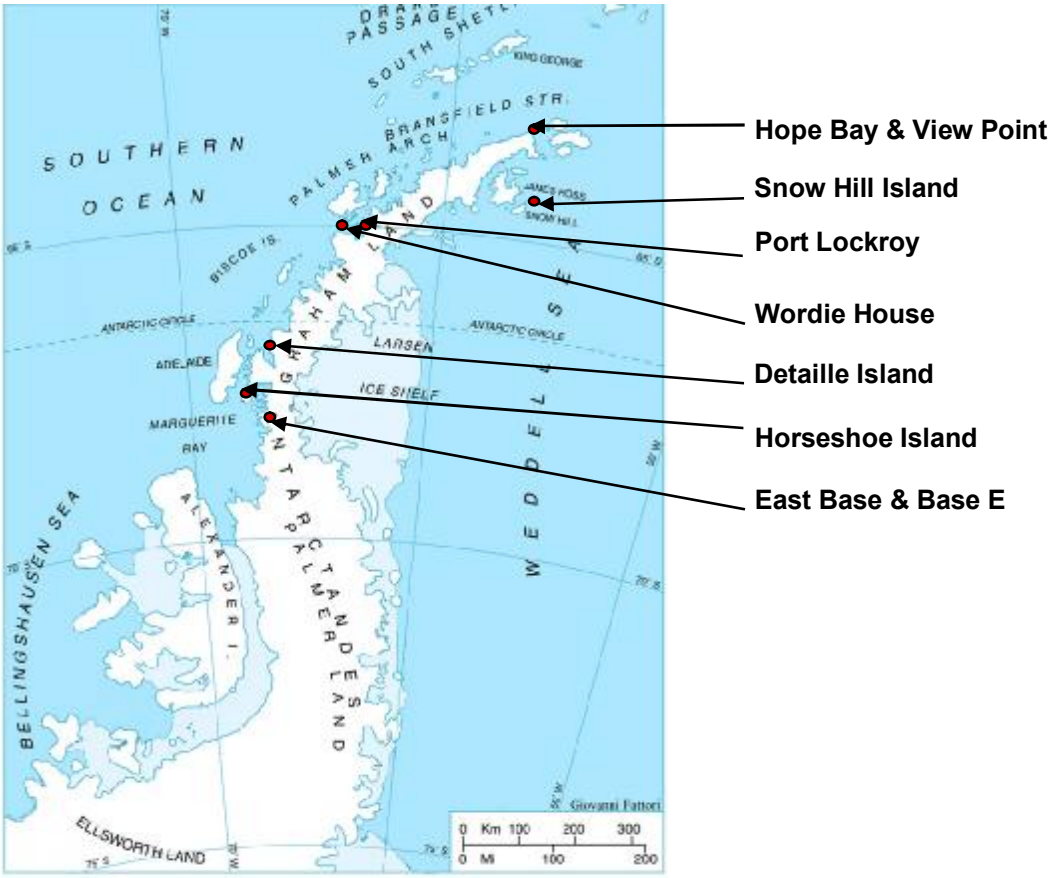

Figure 1.1. Map of the sampling locations on the Antarctic Peninsula.

Figure 1.2. Stonington Island, Base E (British) in foreground, East Base (US) in background.

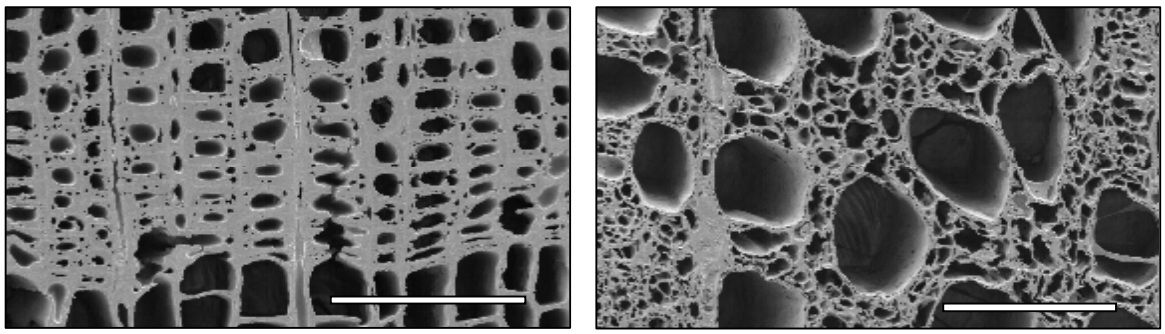

Figure 1.3. (Left) Scanning electron micrograph of soft rot in wood collected from Wordie House. Fungi have attacked the cells causing cavities throughout the secondary cell wall layer. (Right) Soft rot occurring in birch (*Betula sp*.) collected at East Base. Fungi have caused extensive erosion of secondary cell walls as well as cavity formation. (Bars = 100ųm)

Figure 1.4. Exterior wall of former bunkhouse at East Base, with an abundance of dislodged and deteriorated wooden slats.

Chapter 2

Fungal colonization of exotic substrates in Antarctica

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 added 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. Location of plots had a significant effect on fungal counts, with the Palmer Archipelago sites being generally higher; however, the Ross Island plot at Hut Point had the highest fungal counts of all seven plots. Fungal abundance on soil adhering to substrates was found to be similar to that found in non-polar soils indicating that lack of organic matter 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.

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 evidence that Antarctic fungi are able to utilize introduced wood and other organic materials. Several recent 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 sites are associated with the "Heroic Era" of Polar Exploration from 1901-1914 including those on Ross Island left behind 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 temperatures above 0ºC and relative humidity above 75%, occurred for many weeks per year inside the historic huts on Ross Island.

Fungal diversity 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

and yeast ascomycetes, basidiomycete 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 very 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 and 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 on the Peninsula. 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 (Tokura 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 meters 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 and on Anvers Island, Humble Island, and at the Old Palmer Station site in March, 2005. 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 x 6 meter homogenous area of open soil free of large rocks.

Bait traps consisted of 1.5cm x 0.5cm x 10cm pieces of 3 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 into synthetic mesh bags. These four substrates were placed out in an alternating arrangement in 8 rows of 12 samples for a total of 96 pieces per plot (Fig. 2.1). 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 in an arrangement depicted in Figure 2.1 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. Plots were marked by a sign and outlined by a rock arrangement to prevent human disturbance.

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 Backyard site and the Humble Island site were removed for analysis. In March, 2009, the 4 year samples from the Backyard 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 the corners 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 1 in 10 to a maximum of 1 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 20ºC and 4ºC 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 7M 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 15000 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 resuspended 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 hour 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).

Statistical analyses

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 year 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. 2.2). There was a high incidence of sterile substrate-soil samples at the Ross Island plot sites at Cape Royds and Cape Evans where 91 percent of Cape Evans and 77 percent 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 CFU's 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 2.1), 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. 2.2). 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, Backyard, and Limitrophe locations (Fig. 2.3). Differences between fungal counts of substrate-associated soils and background soils at the Cape Royds, Cape Evans, and Humble Island locations were not 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. 2.4, Fig. 2.5) (likelihood ratio test; $P = 0.12$). There was also no significant difference between CFU counts on 2 year and 4 year samples in the locations where 4 year samples were obtained: Backyard, Hut Point, Cape Evans, and Cape Royds (Fig. 2.6) (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 operational taxonomic units (OTUs) were identified in this study based on their unique sequences (Table 2.2). 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%).

Substrate observations

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 (RD) and those of Cape Evans and Cape Royds (Fig. 2.2). 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 ($9.8x10^8$ 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 fungal species 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 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 *Cadophora* genus has an established record of attacking wood and causing a soft rot throughout ice-free regions of Antarctica (Arenz et al. 2006, Arenz and Blanchette 2009, Blanchette et al. 2004, Blanchette 2010, Held et al. 2005).The two taxa that appeared in the next greatest abundance in Palmer Archipelago plots after *Geomyces* and *Cadophora* (Table 2.2) were difficult to resolve taxonomically based on GenBank comparisons. The third most common group (6.9%) had a best Blast match of 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 is it difficult to resolve the potential indigenous/exotic nature of these two groups of fungi. 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 (Vischniac 1985, Vishniac and Hempfling 1979, Vishniac and Hurtzman 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 logCFU 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 2.2). 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 (Hillebrandt 2004). There is also a substantial difference in climate between these two locations. While both are located at coastlines, the Palmer Archipelago sites are influenced by a more typical maritime climate with warmer temperatures, -3°C 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 the Backyard area behind 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 the Cape Evans and Cape Royds Huts, whereas Discovery Hut at Hut Point had relatively less fungal activity in the structure (Arenz et al. 2006, Blanchette et al. 2004, Blanchette et al. 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 in regards 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 2.1). All of these factors were associated with greater fungal abundance based on a wide-ranging companion soil study (Arenz and Blanchette 2010) (Chapter 3 in this thesis).

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 CFU's 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. 2.4). 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 idea that these fungi are primarily restricted by nutrient limitations in their natural soil environments.

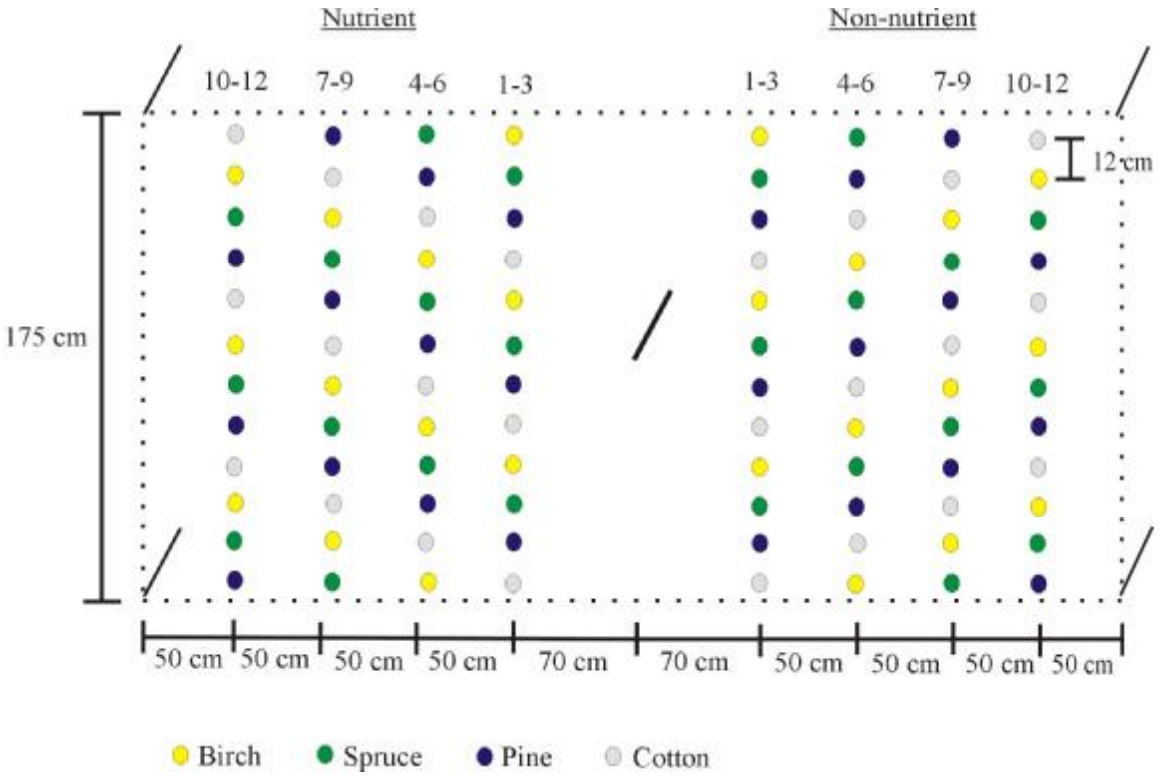

Figure 2.1. Diagram of plot layout used at each of the seven locations in this study. Sterile substrates of different species of wood or cellulose represented by colored circles were buried at a depth of 5 cm.

Table 2.1. 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	1.4	8.30	2701	0.010	0.068	7524		
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	42.7	4.98	363	1.416	16.161	1567808		
Backyard	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

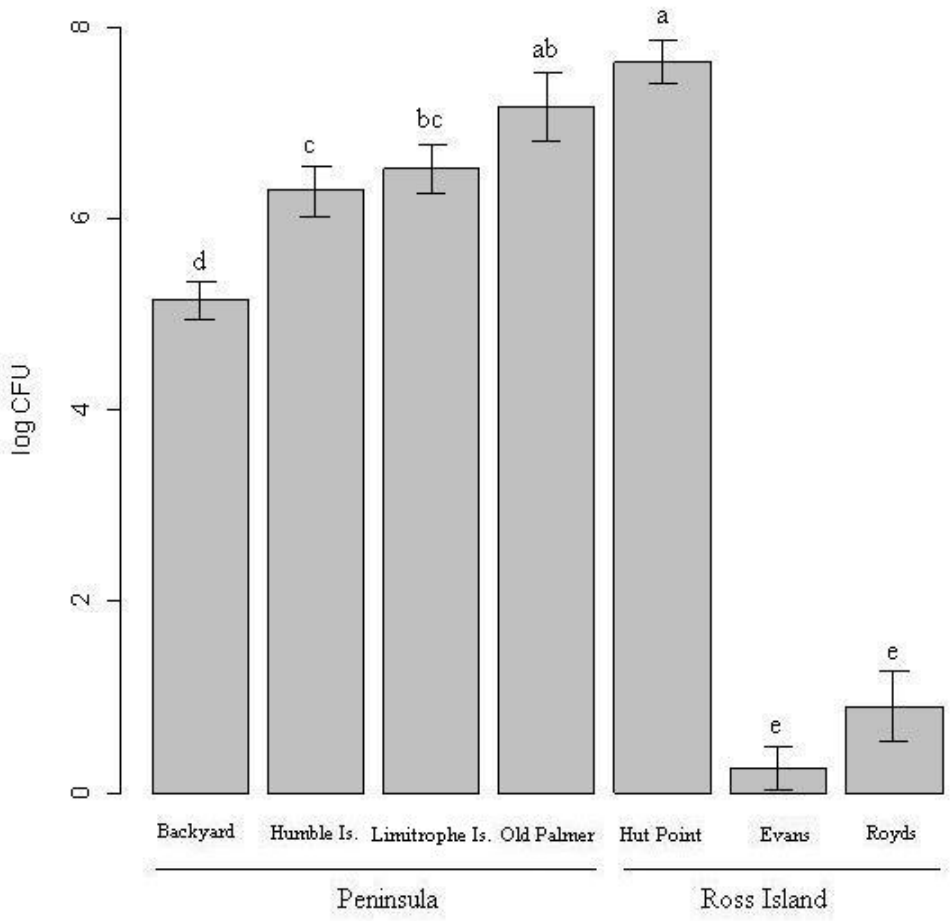

Figure 2.2. Log fungal Colony Forming Unit (CFU) counts by location from seven sites in Antarctica with 95% confidence interval. Significance classes were obtained with a Dunnett-Tukey-Kramer (DTK) Pairwise Multiple Comparison Test.

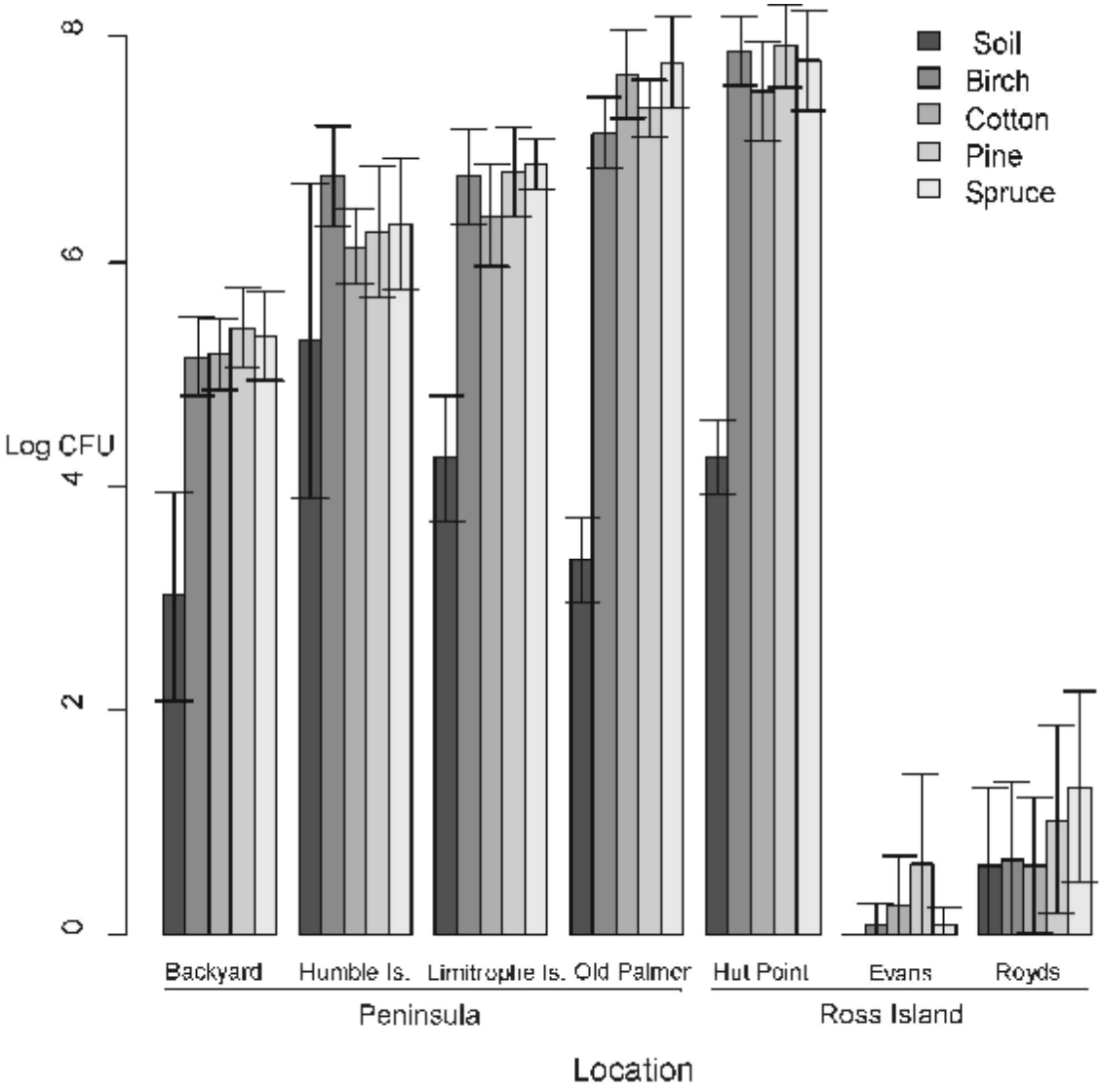

Figure 2.3. Log fungal Colony Forming Unit (CFU) counts from soils associated with various substrates and location of study plot in Antarctica along with the 95% confidence intervals.

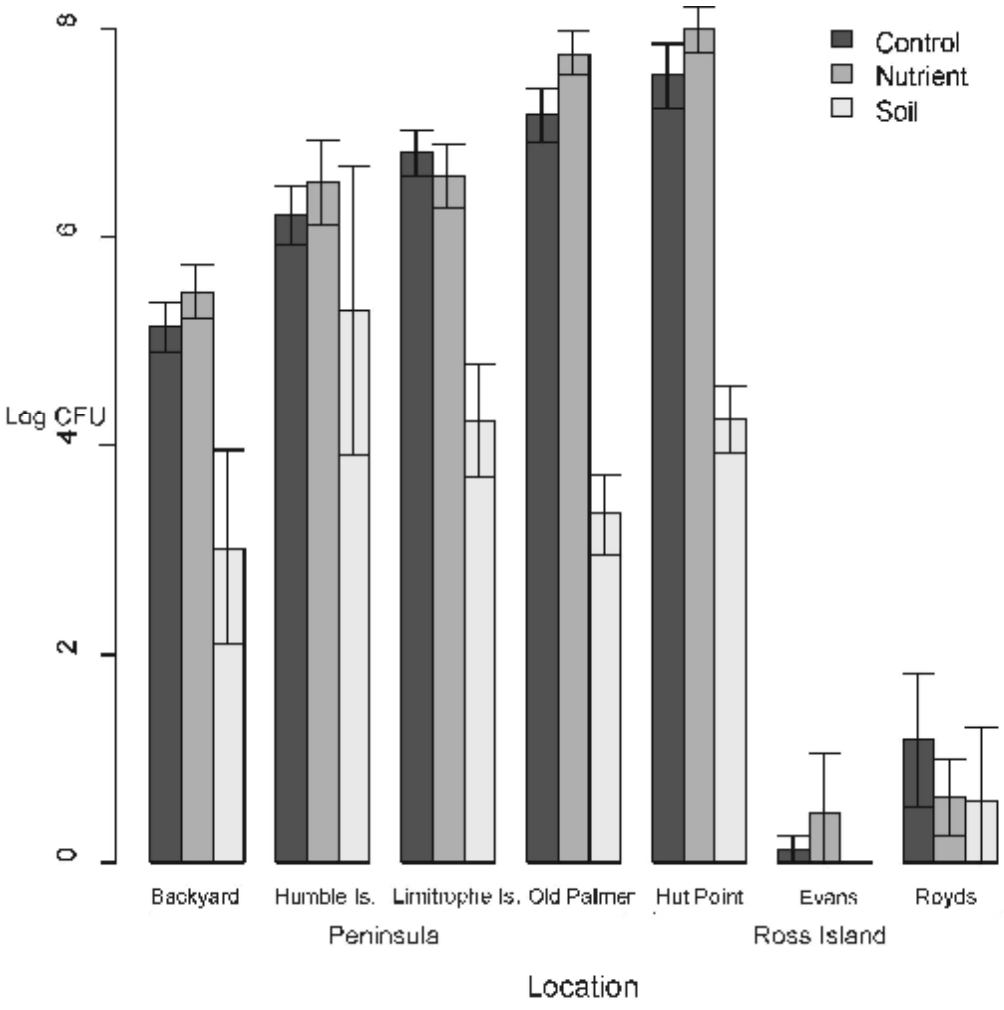

Figure 2.4. Log fungal Colony Forming Unit (CFU) counts of soil associated with substrates with nutrient addition (nutrient) and without (control) and background soil (soil) and locations with 95% confidence interval.

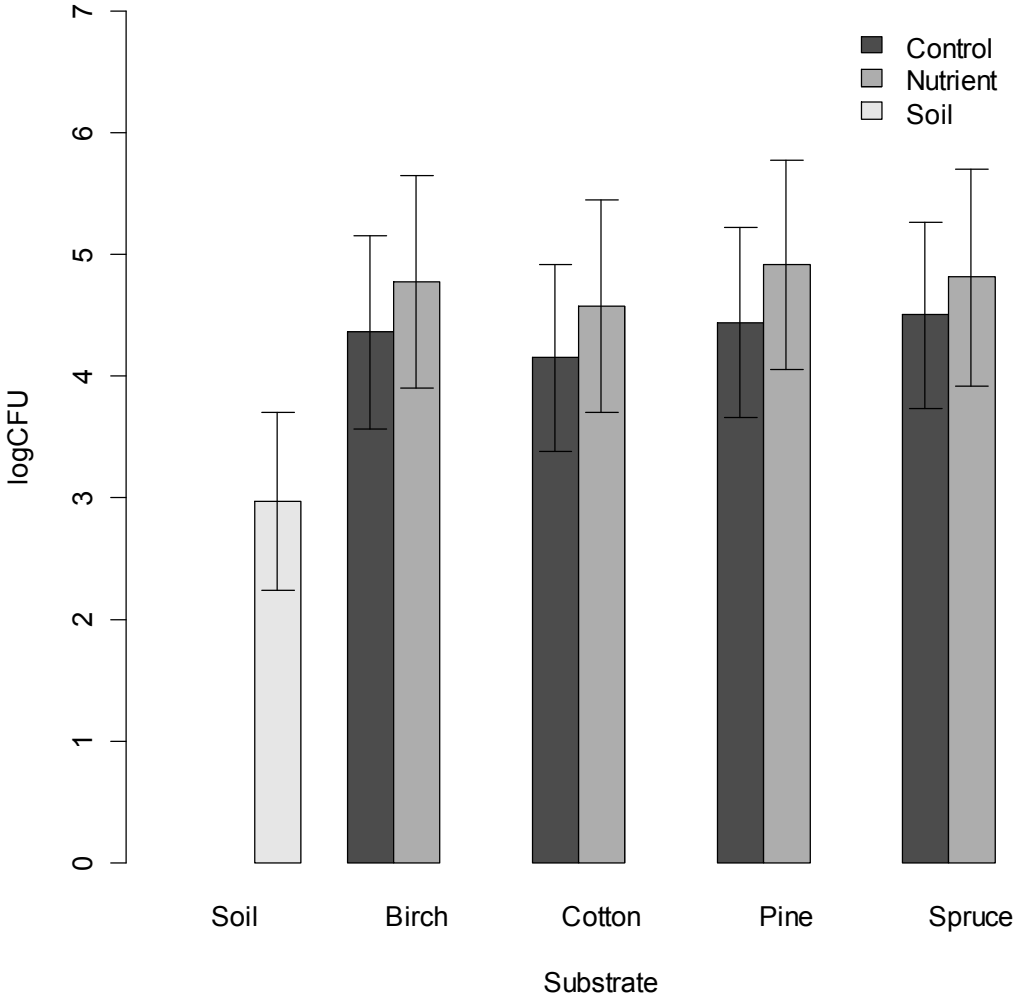

Figure 2.5. Log fungal Colony Forming Unit (CFU) counts of soil associated with substrates by type and nutrient addition (control vs. nutrient) and background soil (soil) with 95% confidence interval.

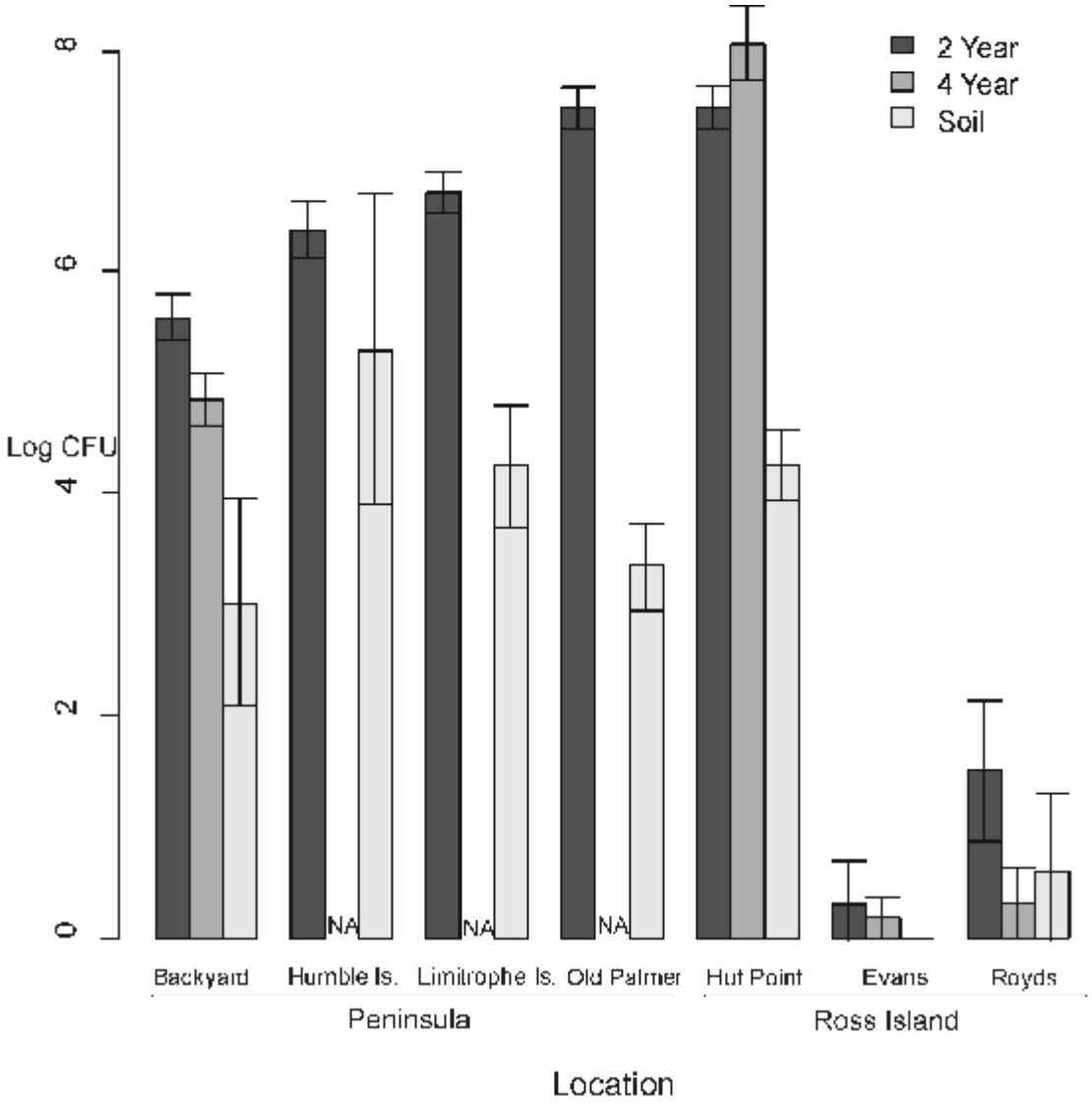

Figure 2.6. Log fungal Colony Forming Unit (CFU) counts of substrate associated soil by length of trial (2 or 4 year interval) and background soil (soil) and location with 95% confidence interval. Humble Island, Limitrophe Island and Old Palmer did not have 4 year samples (NA).

Table 2.2 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 %	Peninsula %	Accession Number
Ascomycete						
Antarctomyces psychrotrophicus [AM489755]	100	502/502	1		0.001	HM589308
Ascomycete sp. BC15 [DQ317348]	99.80	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
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. 04M 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

Chapter 3

Distribution and abundance of soil fungi in Antarctica at sites on the Peninsula, Ross Sea Region and McMurdo Dry Valleys

Fungal abundance and diversity were studied from 245 soil samples collected in 18 distinct ice-free locations in Antarctica including areas in the McMurdo Dry Valleys, Ross Sea Region, and the Antarctic Peninsula. Fungal presence and abundance in soil were found to be most consistently correlated with percent carbon and nitrogen based on multiple regression analysis. Soil moisture, salinity, and pH had less consistent correlations which varied by location. These results suggest that nutrient limitations in these highly oligotrophic environments are a primary factor in determining the distribution and abundance of indigenous fungi. Other effects of the extreme Antarctic environment likely affect fungi indirectly by limiting the distribution and abundance of plant-derived sources of carbon.

Introduction

More than 99% of the continent of Antarctica is covered by ice year-round (Fox and Cooper 1994). Areas free of ice include the McMurdo Dry Valleys and scattered locations around the coastal margin of the continent and nearby islands that are free of ice and snow during the Austral summer. The few exposed soil ecosystems are generally characterized by low microbial abundance and diversity relative to temperate locations. These two characteristics are commonly attributed to the environmental extremes present including low temperature, minimal soil moisture and organic matter, and high salinity and UV radiation.

Antarctic megafauna (seals, penguins, skuas, etc.) occupy terrestrial habitats for defined periods of time and are not considered year round inhabitants of continental

Antarctic (with the possible exception of breeding Emperor Penguins). Permanent terrestrial heterotrophs are limited to microscopic animals (nematodes, rotifers, tardigrades, springtails and mites) and bacteria, protists and fungi. Abundance and diversity of Antarctic terrestrial biota has been found to be primarily determined by abiotic factors (Hogg et al. 2006) with few well documented effects from species-species interactions such as competition or predation.

Fungi appear to play a variety of ecological roles in Antarctica. They are mycobiont partners of lichen symbiosis which in many areas of Antarctica are the only visible evidence of primary production, although they do not support populations of large herbivores as they do in the Arctic (Lindsay 1978). Mycorrhizal associations appear to decrease with increasing latitude but have been found as far south as Livingstone Island (62'38S) in the Maritime Antarctic in the form of arbuscular mycorrhizae on the roots of the Antarctic grass, *Deschampsia antarctica* (Upson et al. 2008). Mycorrhiza-like associations have also been reported on liverworts as far south as Granite Harbor (77'00S) (Williams et al. 1994). In addition, potential plant pathogens such as *Pythium* spp. have been identified on *D. antarctica* in the Maritime Antarctic (Bridge et al. 2008) and pathogens of non-vascular plants including moss, liverworts, and lichenicolous fungi have also been isolated in Antarctica (Pegler et al. 1980, Olech and Alstrup 1996). Nematode trapping fungi have been reported from Antarctic soils (Gray and Lewis Smith 1984) as well as a fungal predator of rotifers and tardigrades (McInnes 2003). Lastly, and potentially most important, is the role of decomposer fungi in Antarctic ecosystems which most indigenous species of fungi isolated from the region are assumed to be (Adams et al. 2006). A functional gene microarray survey of the Antarctic Peninsula

reported a high rate of detection of fungal decomposition genes and suggested that fungi were the dominant decomposers in the Antarctic (Yergeau et al. 2007). Despite the relative importance of this role to carbon and nitrogen cycling in the ecosystem, little is known about fungal decomposition processes in Antarctica except that filamentous basidiomycetes appear to have little presence or involvement in contrast to their relatively important role in decomposition in other parts of the world (Ludley and Robinson 2008).

Antarctic ice-free locations differ significantly in the source and amount of potential resources for utilization by fungi. Exposed soil ecosystems at coastal areas on the Antarctic Peninsula and Ross Island are heavily affected by maritime influences. Although Antarctic terrestrial systems are characterized by low abundance and activity of primary producers, the cold Southern Ocean has relatively high levels of productivity. A small percentage of this fixed carbon is deposited along the coastal zones with the highest concentrations in the ornithogenic soils at penguin rookeries (Tatur 2002).

The Dry Valley soil ecosystems located inland from the ocean are largely exempt from exogenous inputs of nutrients. Notable exceptions include the discovery of mummified seal carcasses up to 40 kilometers from the sea (Barwick and Balham 1967) and the subsequent creation of microhabitats in otherwise oligotrophic soils (Cary et al. 2010). This area is considered a relatively "closed" ecosystem with limited outside influences and primary production in most open soil areas is largely restricted to cryptoendoliths (de la Torre 2003) and hypoliths. Ancient lacustrine sources of fixed carbon originally formed when Dry Valley lake levels were higher also appear to play a role in increasing the soil organic matter pool (Barrett and Wall 2006, Burkins et al.

2000, Virginia and Wall 1999) in addition to allochthonous wind redistribution from contemporary lacustrine sources (Hopkins et al. 2006).

Primary production in coastal areas including Ross Island and the Antarctic Peninsula is largely from the activity of lichens, free-living algae and non-vascular plants such as mosses and liverworts. Only two species of vascular plants are native to the Antarctic and they occur on the Antarctic Peninsula: *D. antarctica* and *Colobanthus quitens* which are found as far south as 68º (Lewis-Smith and Poncet 1985). Analysis of two Peninsula sites using a range of methods (DGGE, ergosterol, real time PCR, and CFU counts) found "fungal abundance was generally higher in vegetated plots" (Yergeau et al. 2007) although the different methods of detection utilized were not entirely in agreement.

The objective of the present study was to compare patterns of soil fungal distribution, abundance and species richness between three major ice-free regions in Antarctica: coastal areas and islands in the Antarctic Peninsula, Ross Island in the Ross Sea Region, and the McMurdo Dry Valleys. Soil characteristics including moisture, salinity, pH, percent carbon, percent nitrogen, and carbon/nitrogen ratio were also analyzed for correlations with fungal abundance.

Materials and Methods

A total of 245 soil samples were obtained from 18 separate locations in Antarctica (Table 3.1). These locations included one from the McMurdo Dry Valleys (McKelvey Valley), five from the Ross Sea area (Cape Evans, Hut Point, Cape Royds from Ross Island and Cape Hallett and the ridge on the Adare Peninsula), and 12 locations on the

Antarctic Peninsula (Detaille Island, Deception Island, Stonington Island, Humble Island, Hope Bay, Horseshoe Bay, Goudier Island, Snow Hill Island, Duse Bay, Winter Island, and areas around the current U.S. Palmer Station and the old Palmer Station site) (Figure 3.1). Peninsula samples were collected in March 2007 while traveling with the British Antarctic Survey (BAS) on the HMS Endurance. Dry Valley samples were collected in January 2008 and Ross Island samples in January 2006 and 2008.

At each location, soil samples were chosen that represented a diversity of geomorphic features. Samples of soil at a 0-5cm depth were collected using sterile scoops, placed in Whirl-Pak (Nasco, Ft. Atkinson, WI) bags, and kept at 4°C until transported to the lab at the University of Minnesota where they were kept at -20°C while awaiting analysis.

Soil was passed through a sterile 3 mm sieve for homogenization and to remove large particles before analysis. Analyses of soil properties were conducted using established methodology (Rhoades 1996, Thomas 1996). Briefly, soil moisture was determined by weighing subsamples before and after 2 hours of oven drying at 120°C. Soil pH was determined by preparing a 1:1 soil/distilled H_2O solution and measurement with an Accumet Research AR15 pH meter (Fisher Scientific, Pittsburgh, PA). Relative soil salinity was determined by measuring soil conductivity based on a 1:5 soil/distilled H_2O solution analyzed with an Orion model 122 conductivity meter (Orion Research Inc., Cambridge, MA. Percent carbon and nitrogen content were measured with a Costech ECS 4010 CHNSO analyzer (Costech Analytical, Valencia, California).

Fungal colony-forming units (CFUs) were determined by making serial dilutions of soil and sterile distilled H_2O. One milliliter aliquots from a range of dilution series

(1/10 to 1/100,000) were plated on both 1% Malt Extract Agar and a basidiomycete select agar (BSA) (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 either 20°C or 4°C for one week after which CFU's were counted and sub-cultures made of all morphologically distinct colonies from each sample. Plates that had no visible CFU's after one week were kept for at least two months to ensure slow growing isolates were not excluded.

Subcultures were incubated for another week and DNA extraction of individual isolations followed, based on an adapted chloroform extraction procedure (Chapter 2 of this thesis). Nuclear rDNA of the Internal Transcribed Spacer (ITS) regions 1 and 2 along with the 5.8S region was amplified via PCR using the primers ITS1 and ITS4 (White et al. 1990). PCR products were sequenced at the University of Minnesota BioMedical Genomic Center (BMGC). Contigs of both forward and reverse sequence were constructed and sequences were compared to the NCBI GenBank database using a BLASTn search.

Statistical analyses and evaluation of relationships between soil characteristics and fungal CFUs were performed with the program R, version 2.9.2 (R Development Core Team, 2009). Regressions used log transformed values of CFU (log (1+ CFU)) as a dependent variable.

Results

From the 245 soil samples included in this study, 76 were found to yield no fungal CFUs on the media used. Although these samples were likely not sterile, they will be considered as such for the purposes of these results. The highest frequency of samples with no CFUs were obtained from the Dry Valley and Ross Island locations (78% and 42%, respectively) compared to few (9%) samples from the Antarctic Peninsula locations. From the remaining 169 soil samples, 455 fungal isolations were made. From these, 306 yielded high quality sequence results and were analyzed for probable taxonomic identity (Table 3.2). Ninety-two unique operational taxonomic units (OTUs) were identified based on rDNA BLASTn results.

Species richness was highest in Peninsula samples (80 OTUs) followed by the Ross Sea region (23 OTUs) and Dry Valley samples (3 OTUs). There was a limited amount of overlap between the locations (Fig. 3.2) with only 1 OTU being isolated from all three major areas. Although there was substantial variation within and among Peninsula fungal counts in general, they tended to be higher than those at the Dry Valleys or Ross Sea Region (Fig. 3.3).

One of the effects of the high incidence of soil with no fungal CFUs was that it gave the dependent response variable (fungal CFUs) a distinctly non-normal distribution when all samples were analyzed together (Fig. 3.4). In response to this, the overall correlation between soil properties and fungal abundance was analyzed in a two part fashion. The first stage was to use multiple logistic regression to evaluate the correlation of soil properties on fungal presence vs. absence. The second stage was to use multiple

linear regression to determine the correlation response with fungal abundance amongst only the samples that yielded fungal CFUs.

The soil variables of moisture, conductivity, percent carbon, percent nitrogen, and C/N ratio tended to be significantly right-skewed (Fig. 3.4). In order to increase symmetry of the data and improve accuracy of statistical analysis these variables were log-transformed (Fig 3.5). Removal of samples which yielded fungal growth resulted in a dataset with variables that were fairly normally distributed aside from a slight bimodal conductivity distribution and a small secondary peak in logC and logN (Fig. 3.6).

All locations analyzed by simple regression

Moisture, percent carbon, percent nitrogen, and C/N ratio are all positively correlated with the log transformed CFU's measure of fungal abundance (Table 3.3). Electrical conductivity (representing salinity) and pH were negatively correlated with fungal abundance. As previously stated, the assumption of normal distribution was not met for this data set so the results of simple regression should be interpreted with caution. However, these correlations were also supported by principal components analysis (Fig. 3.7). In addition, soils with no fungi had significantly lower soil moisture, percent carbon and nitrogen, C:N ratio and significantly higher conductivity and pH then soils with fungi ($p<0.05$) (Table 3.4).

All locations analyzed by multiple logistic and linear regression

The presence of culturable fungi in samples was significantly positively correlated with soil moisture ($p < 0.05$), percent nitrogen ($p < 0.01$), percent carbon ($p<0.01$) and

C:N ratio ($p< 0.05$) and negatively correlated with soil conductivity ($p<0.001$). In addition, the interaction effect of pH and C:N ratio was also significant ($p<0.05$) with pH being negatively correlated with fungal presence when the C/N ratio was high but having no significant effect when the C/N ratio was low. When only samples which yielding fungal CFU's were considered by multiple linear regression, percent carbon and percent nitrogen were positively correlated with fungal abundance ($p <0.001$, $p <0.01$ respectively) as well as the C:N ratio ($p <0.001$) (Fig. 3.6). The interaction effect of percent nitrogen and C:N ratio was also significant ($p<0.01$) with nitrogen having a greater positive correlation with fungal abundance when the C:N ratio was high then when it was low.

Specific locations analyzed by multiple logistic and linear regression

No soil factors were found to be correlated with presence vs. absence of fungi or abundance of fungi among the present samples when only Dry Valley samples were considered. However, the low number of data points from this sample site (only 8 out of 37 samples produced fungal colonies) may be partly responsible for the lack of significant correlation. At Ross Sea sampling sites none of the measured soil factors were correlated with the presence of culturable fungi. When only fungal-yielding samples are considered, both percent carbon and nitrogen had a significantly positive correlation with fungal abundance ($p < 0.05$). Among Peninsula samples both percent carbon and nitrogen are significantly positively correlated with fungal presence ($p<0.01$, $p<0.05$ respectively). Among samples with fungal presence, there were three significant interaction effects, pH:logmoisture , pH:logconductivity, and logN:logC/N ratio that were correlated with

fungal abundance. pH was positively correlated with log CFU's and had a greater effect when moisture was high ($p < 0.02$). Conductivity had no significant effect on log CFUs when pH was low but was negatively correlated when pH was high ($p < 0.02$). Percent nitrogen was positively correlated with log CFUs and had a greater effect when the C/N ratio was also high ($p < 0.05$).

Taxa found

All three major location areas were predominately represented by ascomycetes in both CFU abundance (94.5% Peninsula (P), 69% Ross Sea Region (RSR), 53% Dry Valley (DV)), and frequency of isolation (65.4% P, 53% RSR, 80% DV). The Peninsula had the highest zygomycete CFU abundance component (2.8% P, 0.03% RSR, 0% DV) and isolation component (12.6% P, 1.4% RSR, 0% DV). The Ross Sea Region and Dry Valleys had the most significant basidiomycete CFU abundance component (2.6% P, 30.8% RSR, 46.8% DV) and isolation frequency component (22% P, 45.7% RSR, 20% DV). OTUs were placed into genera based on a 95% identity BLASTn match critera. The fungal genus isolated with the highest total CFU abundance was *Geomyces* from the Ross Sea Region and Peninsula locations (61% and 54% respectively) (Table 3.2). *Cadophora* spp. represented 22% of total CFU abundance from the Antarctic Peninsula and 1.9% from Ross Sea Region. The basidiomycetes yeast genera *Rhodotorula* and *Cryptococcus* made up 21% and 9.8% of Ross Sea Region total CFU abundance and 1.7% and 0.9% from the Peninsula, respectively. The single oomycete isolate found (best blast match: Uncultured Pythium R021) was from a sampling site on the Antarctic Peninsula (Humble Island).

Discussion

The predominance of filamentous ascomycetes in studies of Antarctic soils is typical and the most abundant ascomycete genera found in this study, *Geomyces* and *Cadophora,* are widely reported from the Antarctic (Bridge et al. 2009) and an indigenous status for these fungi seems likely. These genera also seem to be particularly well-suited to exploit exotic substrates introduced by humans (Chapter 2 in this thesis) as their presence is widely noted in studies of fungal diversity of historic sites (Arenz and Blanchette 2009, Arenz et al. 2006, Blanchette et al. 2010, Blanchette et al. 2004, Held et al. 2005). Based on their association with a wide variety of organic materials in the Antarctic including lichen and moss (Moller and Dreyfuss 1996; Tosi et al. 2002) wood, leather and paper (Arenz et al. 2009), feathers (Marshall, 1998; Del Frate and Carretta 1990), cotton (Arenz et al. 2010), and a mummified seal carcass (Greenfield 1981), these genera likely play important roles as generalist decomposers in the Antarctic soil environment.

The exclusive use of culture based fungal detection techniques limits the conclusions that can be drawn from this study. These techniques undoubtedly biased fungal abundance measurements towards species of fungi which readily grow on either of the culture media used here as well as species that had greater rates of sporulation. Furthermore, many species of "unculturable" fungi have been detected only by use of molecular techniques (Jeewon and Hyde, 2007) and would not be represented in this study. However, previous research on soils from the Ross Sea area using similar media types as well as the molecular detection technique of denaturing gradient gel

electrophoresis (DGGE) found that approximately 2/3 of total taxa could be detected using culturing-based investigations (Arenz et al. 2006).

Many of the sampling locations in this study are also sites that have had significant past or present human activity. The reason for this bias is that soil sampling for this study was done in conjunction with related studies on fungal diversity and deterioration effects at historic locations in the Antarctic (Arenz et al. 2010 (Chapter 2 in this thesis), Arenz and Blanchette 2009, Arenz and Blanchette 2006). The two sites with the most significant current human impacts were Hut Point (within 200 m of McMurdo Station) on Ross Island and the Palmer site (within 100 m of Palmer Station) on the Antarctic Peninsula. All other sampling sites (except McKelvey) were relatively close to historic Antarctic sites and likely affected by past human inputs in the form of fungal introductions and influence on local soil organic matter content. The one exception to this was the sampling site at McKelvey Valley in the McMurdo Dry Valleys, which can be considered a relatively pristine site with little human influence.

Carbon and nitrogen

Carbon and nitrogen concentrations in soil, which are strongly positively correlated ($R^2 = 0.72$), are directly reflective of abundance of primary producers present in the immediate environment or allocthonous inputs from other locations. In the Antarctic, this is typically from penguin rookeries on the coastland. However, at many of the sites in this study the allocthonous input was by past and present activities of humans and the transport of wood, food stuffs, textiles, fuels, and other materials relatively high in carbon and/or nitrogen content (Arenz et al. 2009). Whatever the source of these

materials, a consistent positive correlation between high carbon and nitrogen percentages and fungal abundance was found in this study. A positive carbon/fungal abundance relationship was also noted in Dry Valley soils by Connell et al. (2006). Malosso et al. (2004) found significant increases in both soil respiration and fungal biomass, as measured by ergosterol, when shredded *D. antarctica* leaves were added to soil collected from the Antarctic Peninsula. Addition of glucose, glycine, and NH_4Cl in a Dry Valley soil experiment increased soil respiration rates indicating both C and N limitations on the activity of microbial heterotrophs (Hopkins et al. 2006).

C:N ratio

C:N ratios varied significantly among sites (Table 3.1) with very low values (1-2) occurring in McKelvey Valley to high levels averaging in the mid-40's at two sites on the Peninsula. These high values were reflective of the influence of woody substrates brought to these historic sites. Interestingly, the sites with the highest percent carbon (above 10%) had only moderate C/N ratios (from 8 to 13). Nitrogen was found to have a more significant positive correlation with fungal abundance when the C:N ratio was also high, which tended to be in Peninsula soils where vegetation levels were more significant. Nitrogen limitations have been linked to limiting decomposition of plant-based materials (Jinggua and Bakken, 1997) and Antarctic soil ecosystems may respond in a similar way. Significant nitrate content in very old Dry Valley soils (low C:N ratios) from long periods of atmospheric deposition may be preventing nitrogen from being a limiting factor in these areas (Barrett et al. 2006).

Moisture

When evaluated with simple regression, moisture was found to be positively correlated with fungal log CFU's (R^2 = .11, p-value < .001), and multiple logistic regression found moisture also to be significantly correlated with fungal presence. However, moisture was not found to be significantly correlated with fungal abundance based on multiple linear regression, which is surprising as moisture has been described as the primary biological limiting factor in Antarctic soils (Kennedy 1993) and has been correlated with fungal abundance in Dry Valley sites (Connell et al. 2006). A possible explanation for this discrepancy is that of all the variables included in this study (moisture, salinity, pH, C, N), moisture is the soil characteristic that would vary the most on a temporal scale. The same location could have relatively high moisture for part of the year due to periodic meltwater availability but be dry at other times. Fungal activity will likely fluctuate rapidly during these times but overall abundance may not change drastically as fungi could survive the dry periods in a dormant state or as spores. Moisture was not found to be significantly correlated with bacterial abundance in areas around Wilkes Land, Antarctica with the same possible explanation being discussed (Heatholfe et al. 1989).

A related factor that could be masking a possible moisture effect is that moisture and C and N levels are highly correlated (R^2 = 0.48, p-value = 0.02 and R^2= 0.5, p-value <0.001 respectively). Higher moisture levels and more frequent precipitation are likely supporting a greater abundance of primary producers, which would in turn provide C and N to soil fungi, and so moisture may be influencing fungi more indirectly than directly. Moisture has been found to be significantly positively correlated with microalgal

colonization in Antarctic soil (Wynn-Williams et al. 1997). Another explanation for this relationship could be the that more highly vegetated sites have a much higher water holding capacity than the mineral soils at Ross Island and in the Dry Valleys. Humble Island, Detaille Island, Palmer Station, and Hope Bay contained a lot of vegetation with species of moss as well as the two Antarctic vascular plants, *D. antarctica* and *C. quitens*. This vegetation level is reflected in the high soil C and N concentrations at these sites (Table 3.1) and the very high percentages of soil moisture that were observed there. The predominantly mineral soils at the Dry Valleys and Ross Island and other Peninsula sampling locations simply cannot reach these high levels of moisture even at saturation levels. In addition to capacity for much higher maximum moisture levels at these vegetated sites, the soils have a greater ability to retain moisture for longer periods of time providing a more stable resource for fungi and other microbes.

Salinity

Conductivity, which is a good indicator of soil salinity, was found to be significantly negatively correlated with fungal presence as well as abundance based on simple regression of all samples (Table 3.3). However, no significant correlation was found with multiple linear regression on abundance among the sample set that included only the samples that produced fungal CFUs. Connell et al. (2006) also found salinity to have no significant correlation with fungal abundance in their Dry Valley sample sites. However, salinity has been found to be negatively correlated with nematode presence and abundance in other studies at the Dry Valleys (Poage et al. 2008). It is possible that an indirect influence (similar to that from moisture discussed previously) is affecting fungi

through effect of salinity on primary producer presence. Salinity is reported to have negative effects on *D. antarctica* (Ruhland and Krna 2010) and is likely influencing the distribution of this grass as well as that of mosses and lichens (Broady 1989).

pH

When all locations were evaluated together, pH was found to be significantly negatively correlated with fungal presence when the C/N ratio was high. This finding conflicts with a previous study from the Dry Valleys which found filamentous fungal abundance to be positively correlated with pH ($R^2 = .39$) (Connell et al. 2006) and a decomposition study from moss communities of Signy Island which implicated low pH values in contributing to very low decomposition rates (Davis 1986). In the present study there were an insufficient number of samples from the Dry Valleys that yielded fungal colonies to provide support or to refute these previous results. Roser et al. (1993) found an increase in fungal dominance of the microbial community with declining pH in ornithogenic soils as well as increases in algal abundance. pH may be associated with distribution of flora in non-ornithogenic soils but it is unclear if it is actively affecting primary producer presence or is merely reflective of overall precipitation at a site. High pH values are typically found in soils with very low precipitation and a negligible leaching effect of alkaline ions. In the study reported here, there was a negative correlation found between pH and soil moisture ($R^2 = 0.22$, $p < 0.001$). More research is needed on possible pH-fungal interactions as pH has been recently shown to be a powerful predictor of soil bacterial community structure on a wide ranging study of ecosystems in North and South America (Lauber et al. 2009). Varying pH in different

areas of soil may provide a competitive advantage for certain fungal species but it is unclear if there is an overall direct effect on fungal abundance.

Conclusions

Constructing a putative model for Antarctic fungal distribution and abundance based on these results would involve direct effects from carbon and nitrogen concentrations and relatively indirect effects from moisture, salinity and pH. This model would support the assumption that the majority of non-lichenized Antarctic fungi are operating as decomposers in their respective ecosystems. *Cadophora* and *Geomyces* spp. seem to be particularly successful generalist decomposers with a relatively wide distribution and diversity. Primary producers provide the main source of bio-available carbon in areas outside of ornithogenic soils and their distribution may be influenced more directly from moisture, salinity, as well as nitrogen. The results from this study support the theory that plant-derived nutrient limitations are a primary factor in determining distribution and abundance of fungi in Antarctic mineral soils, which is similar to the effect found for fungi in other arid regions (Zak 2005).

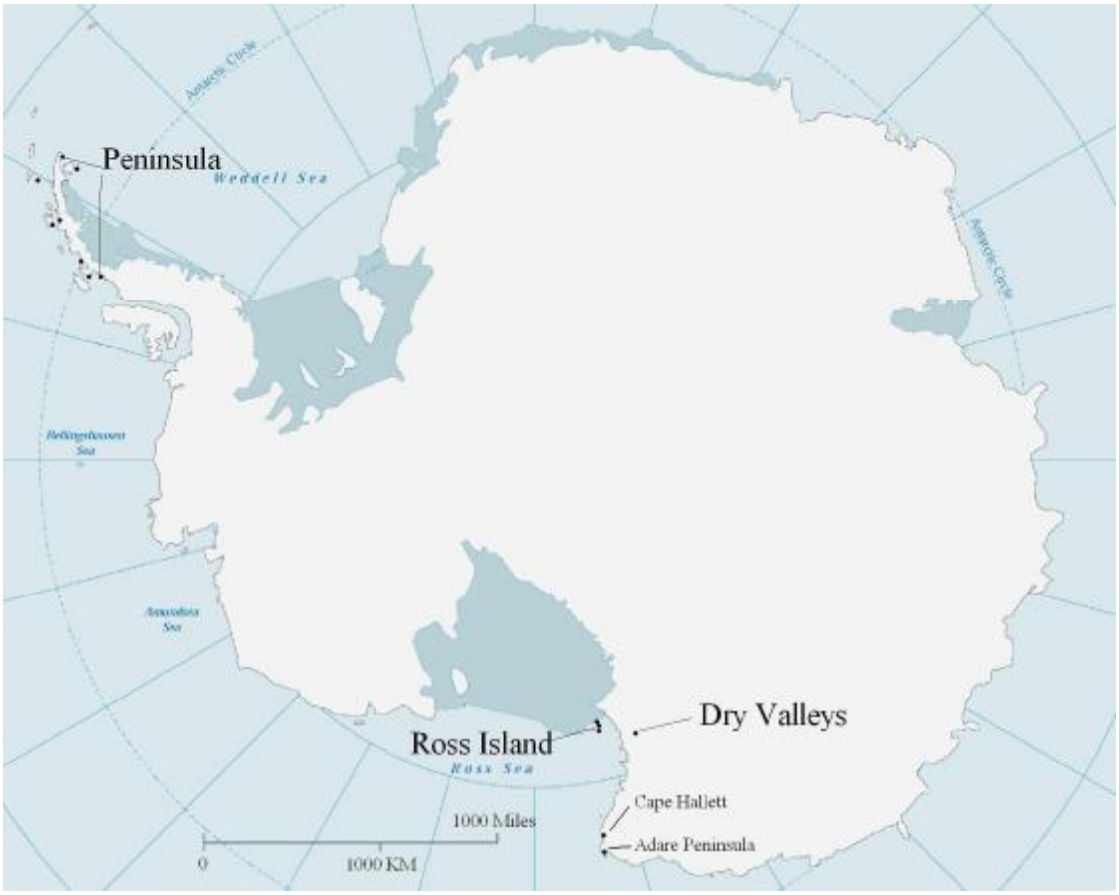

Figure 3.1. Map of Antarctica showing locations of three sampling regions; Antarctic Peninsula, Ross Island, and the McMurdo Dry Valleys. The Ross Sea sampling area also included Cape Hallett and the ridge of the Adare Peninsula in Victoria Land.

Table 3.1. Locations where samples were collected for this study. Number of samples used as well as soil characteristics and average fungal Colony Forming Units (CFUs) per gram of soil is noted.

Location	Sample Count	Moisture%	pH	EC (µS)	N	C	C/N ratio	CFU	Latitude	Longitude
Dry Valleys (McKelvey)	37	2.7	8.45	2806	0.052	0.071	1.35	553	77° 26? S	161° 33? E
Ross Island	82	6.8	8.04	1275	0.026	0.296	11.17	14743		
Hut Point	12	4.3	8.35	1670	0.011	0.087	8.23	26149	77° 50'S	166° 38'E
Cape Evans	39	7.0	7.74	1702	0.034	0.367	10.78	2060	77° 38'S	166° 24'E
Cape Royds	32	7.4	8.29	607	0.023	0.288	12.37	25925	77° 33'S	166° 10'E
Ross Sea	5	3.7	7.56	2091	1.088	4.337	3.99	464		
Cape Hallett	3	2.0	7.09	3398	1.796	7.177	4.00	484	72° 19'S	170° 16'E
Adare Peninsula	2	6.2	8.26	132	0.025	0.076	3.00	671	71° 40'S	170° 25'E
Peninsula	120	20.5	6.25	532	0.588	6.793	11.55	234900		
Detaille Island	5	53.0	6.95	629	2.668	32.723	12.26	775504	66° 52'S	66° 48'W
Deception Island	26	13.8	6.51	577	0.024	0.445	18.73	27711	62° 59'S	60° 34'W
Stonington Island	10	9.9	6.07	1276	0.133	5.518	41.54	135554	68° 11'S	67° 00'W
Humble Island	3	76.7	4.09	1996	3.092	29.373	9.50	367400	64° 46'S	64° 06'W
Hope Bay	11	19.9	5.48	125	1.093	12.773	11.69	161961	63° 24'S	56° 59'W
Horseshoe Bay	19	15.8	6.08	186	0.306	4.899	16.03	467118	67° 49'S	67° 18'W
Old Palmer Site	4	18.9	4.57	80	0.057	0.339	6.00	4056	64° 46'S	64° 05'W
Palmer Station	10	30.7	4.78	62	0.592	7.124	12.03	2647	64° 47'S	64° 04'W
Goudier Island	7	45.1	6.11	1202	2.293	18.824	8.21	1265577	64° 49'S	63° 30'W
Snow Hill Island	11	16.9	8.07	799	0.453	3.025	6.67	16115	64° 27'S	57° 12'W
Duse Bay	7	3.2	7.06	31	0.071	0.505	7.16	181057	63° 32'S	57° 23'W
Winter Island	6	12.3	7.09	608	0.078	3.604	46.06	16539	65° 15'S	64° 16'W

Table 3.2. Fungi isolated with best BLASTn comparisons with NCBI Genbank database. Total number of isolations are noted along with percent of total identified fungal CFU composition per location (RS: Ross Sea Region, DV: Dry Valley, P: Peninsula).

				Locations			
Best Blast	**Percent**	**Overlap**	**# Isolations**	**RS**	**DV**	**P**	**Accession**
Ascomycete							
Alternaria sp. IA317 (EF505090)	100	462/462	1			<0.01	HM589216
Antarctomyces psychrotrophicus (AM489755)	100	502/502	1			0.06	HM589217
Ascomycete sp. BC15 (DQ317348)	100	491/491	8	0.05		<0.01	HM589218
Ascomycete sp. nasa65 (DQ683978)	88.9	433/487	1			0.11	HM589219
Aspergillus sydowii (EF652451)	100	516/516	1			<0.01	HM589220
Aspergillus unguis isolate NRRL 5041 (EF652497)	99.6	523/525	1			<0.01	HM589221
Cadophora fastigiata (DQ317326)	100	546/546	1			<0.01	HM589222
Cadophora luteo-olivacea isolate PhiK3II (FJ486274)	99.6	571/573	1			0.23	HM589223
Cadophora luteo-olivacea isolate PhiK3II (FJ486274)	99.8	580/581	1			<0.01	HM589224
Cadophora luteo-olivacea strain 7R38-4 (GU212374)	100	556/556	1			0.26	HM589225
Cadophora malorum strain 7R73 (GU212434)	100	529/529	10	1.46		0.11	HM589226
Cadophora sp. BDC-22-66e (FJ666350)	98	551/562	4	0.4		0.03	HM589227
Candida glaebosa (FM178351)	90	566/572	1			<0.01	HM589228
Candida mesenterica (FM178362)	100	319/319	1			<0.01	HM589229
Candida sp. K2 (AJ549823)	99.2	381/384	1			<0.01	HM589230
Chaunopycnis sp. ANT 03-065 (DQ402530)	99.8	516/517	4	2.69	50		HM589231
Debaryomyces hansenii strain CBS 940 (DQ249204)	98	559/570	1			0.02	HM589232
Debaryomyces hansenii strain MA09-AK (GQ458025)	100	587/587	2			0.05	HM589233
Debaryomyces hansenii voucher MCCC2E00222 (EF194843)	100	616/616	1	0.76			HM589234
Debaryomyces sp. CBS 5572 (AM992909)	100	583/583	4			0.20	HM589235
Environmental fungal clones (AM113721)	98.8	499/505	1			0.11	HM589236

Exophiala sp. BC36 (DQ317336)	99.6	555/557	1	1.52		HM589237
Fungal endophyte sp. ECD-2008 (EU686037)	99	505/510	2		0.83	HM589238
Fungal sp. AB3 (FJ235936)	100	511/511	1		<0.01	HM589239
Fungal sp. AB32 (FJ235965)	99.8	474/475	7		2.99	HM589240
Fungal sp. AB34 (FJ235967)	100	502/502	13		4.59	HM589241
Fungal sp. AB47 (FJ235980)	100	535/535	3		20.94	HM589242
Fungal sp. AB48 (FJ235981)	98.8	499/505	2		0.23	HM589243
Fungal sp. AB48 (FJ235981)	99.8	507/508	2		0.37	HM589244
Fungal sp. AB56 (FJ235989)	99.8	545/546	5		2.91	HM589245
Geomyces pannorum strain ASIGP1 (DQ779788)	93.3	472/506	4		23.21	HM589246
Geomyces pannorum strain ASIGP1 (DQ779788)	92.9	443/477	1		0.40	HM589247
Geomyces sp. BC7 (DQ317337)	100	503/503	35	35.14	16.98	HM589248
Geomyces sp. BC7 (DQ317337)	99.8	516/517	5		0.24	HM589249
Geomyces sp. BC9 (DQ317339)	100	552/552	2	0.21	6.9	HM589250
Geomyces sp. FFI 30 (AJ608960)	99.6	523/525	11		1.09	HM589251
Geomyces sp. FMCC-3 (DQ499473)	100	514/514	1		<0.01	HM589252
Geomyces sp. T489/9b (AY345348)	99.8	499/500	9	0.45	0.92	HM589253
Helotiales sp. MK9 (EU700254)	97	449/463	1		<0.01	HM589254
Holwaya mucida (DQ257357)	96.6	488/505	5		3.56	HM589255
Hypocrea lixii strain DIS 303A (FJ442646)	100	553/553	1		<0.01	HM589256
Onygenales sp. 7R11-3 (GU212399)	100	507/507	9	25.08	4.59	HM589257
Onygenales sp. 7R19-1 (GU212423)	99.6	511/513	2		0.03	HM589258
Phoma herbarum (DQ132841)	99.6	475/477	3	0.59		HM589259
Pochonia chlamydosporia strain Pt1 (GQ369959)	89.5	479/535	1		0.34	HM589260
Pseudeurotium bakeri clone NS202B (DQ068995)	99.4	517/520	1		0.20	HM589261
Pseudeurotium desertorum CBS 986.72 (AY129288)	95.9	487/508	1		<0.01	HM589262
Stictis radiata isolate MW6493 (AY527309)	82.9	435/525	4		0.12	HM589263
Teberdinia hygrophila CBS 326.81 (AY129293)	99.6	470/472	1		1.26	HM589264
Thelebolaceae sp. BC17 (DQ317350)	100	524/524	1		<0.01	HM589265

Thelebolaceae sp. BC18 (DQ317351)	99.8	526/527	7	0.45	3.16	0.23	HM589266
Thelebolus microsporus (DQ402525)	100	530/530	1	0.38			HM589268
Uncultured ascomycete isolate dfmo0690_022 (AY969478)	99	481/486	1			<0.01	HM589269
Uncultured ectomycorrhiza (Leotiomycetes) clone LTSP_EUKA_P6K14 (FJ554384)	97.6	492/504	1			0.06	HM589270
Uncultured fungus clone G49 (EU620157)	99	520/525	1			0.34	HM589271
Uncultured Tetracladium clone NG_P_E09 (GU055641)	99.4	503/506	1			0.03	HM589268
Basidiomycete							
Cryptococcus diffluens (AM117836)	100	571/571	1	0.07			HM589272
Cryptococcus gastricus (AB032677)	100	590/590	1			0.09	HM589273
Cryptococcus gastricus (AB032677)	99.8	578/579	6			0.07	HM589274
Cryptococcus liquefaciens strain SN1 (FJ515174)	100	573/573	1	0.03			HM589275
Cryptococcus sp. NRRL Y-17490 (AF444449)	100	612/612	5	1.95			HM589276
Cryptococcus sp. YSAR10 (AM922286)	98.6	577/585	1			<0.01	HM589277
Cryptococcus sp. YSAR10 (AM922286)	99	606/612	8			<0.02	HM589278
Cryptococcus victoriae strain CBS 8884 (AF444645)	100	514/514	12	0.03		0.70	HM589279
Fibulobasidium murrhardtense strain CBS9109 (GU327540)	89.3	407/456	1			<0.01	HM589280
Fungal sp. BB12 (FJ236003)	99.5	440/442	5	5.33			HM589281
Fungal sp. BB14 (FJ236005)	100	529/529	9			0.13	HM589282
Fungal sp. BB17 (FJ236008)	100	570/570	2			<0.01	HM589283
Fungal sp. BB5 (FJ235996)	98.5	449/456	10	7.7			HM589284
Fungal sp. BB8 (FJ235999)	100	591/591	1			<0.01	HM589285
Leucosporidiella yakutica VKM Y-2837T (AY212989)	100	554/554	1		46.84		HM589286
Rhodotorula laryngis strain CBS2221 (AF190014)	100	551/551	3	0.06		0.01	HM589287
Rhodotorula slooffiae strain PYCC 4887 (AF444589)	100	443/443	2	0.03			HM589288
Rhodotorula sp. BC22 (DQ317357)	99.8	533/534	12	4.39		0.24	HM589289
Sporobolomyces lactosus isolate C4 (EU551181)	100	464/464	1			<0.01	HM589290
Tremella indecorata HBZ194 (AF042432)	98.8	496/502	1			<0.01	HM589291

Zygomycete						
Fungal sp. ZB2 (FJ236010)	100	592/592	1		<0.01	HM589292
Fungal sp. ZB2 (FJ236010)	99.8	601/602	2		<0.01	HM589293
Fungal sp. ZB5 (FJ236013)	100	604/604	1		0.03	HM589294
Fungal sp. ZB5 (FJ236013)	99.5	604/607	4		0.07	HM589295
Mortierella alpina (AB476415)	100	607/607	2		0.29	HM589296
Mortierella sp. 04M 158 (AY842393)	100	606/606	1		<0.01	HM589297
Mortierella sp. WD35C (EU240119)	99.8	625/626	5		1.41	HM589298
Mortierellaceae sp. BC21 (DQ317354)	99.8	606/607	1		0.06	HM589299
Mortierellaceae sp. BC21 (DQ317354)	99.8	615/616	2	0.03	0.06	HM589300
Mortierellaceae sp. BC21 (DQ317354)	100	605/605	5		0.19	HM589301
Mortierellales sp. WD8I (EF126342)	99.5	614/617	1		<0.01	HM589302
Mucor hiemalis (AJ876489)	99.7	592/594	1		<0.01	HM589303
Uncultured fungus (FN397313)	99.5	622/625	1		<0.01	HM589304
Uncultured *Mortierella* clone LTSP_EUKA_P4O11 (FJ553914)	99.5	579/582	1		0.03	HM589305
Uncultured soil fungus clone TC_fungal-F7-ITSFL (GU083113)	99	585/590	1		0.63	HM589306
Oomycete						
Uncultured *Pythium* Ro21 (AY129553)	99.5	875/879	1		<0.01	HM589307

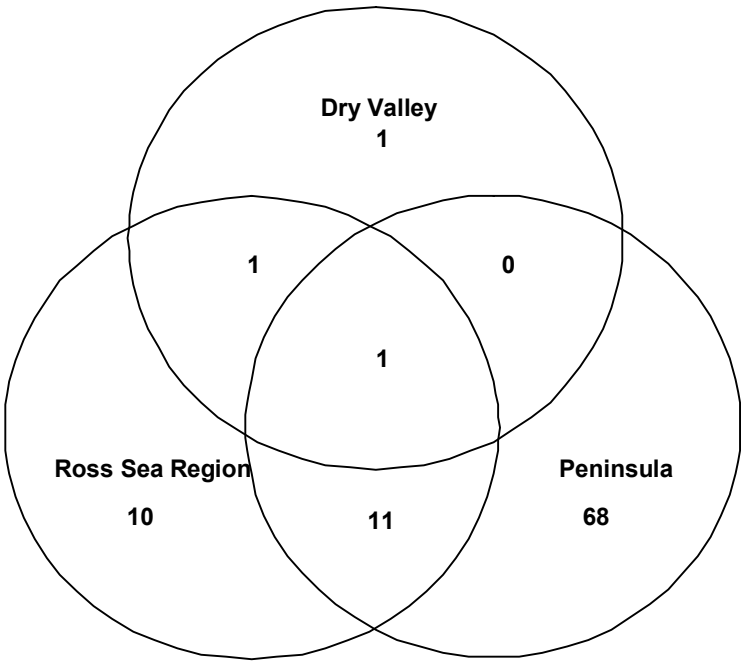

Figure 3.2. Number of unique operational taxonomic units (OTUs) based on ITS rDNA sequence data found by location.

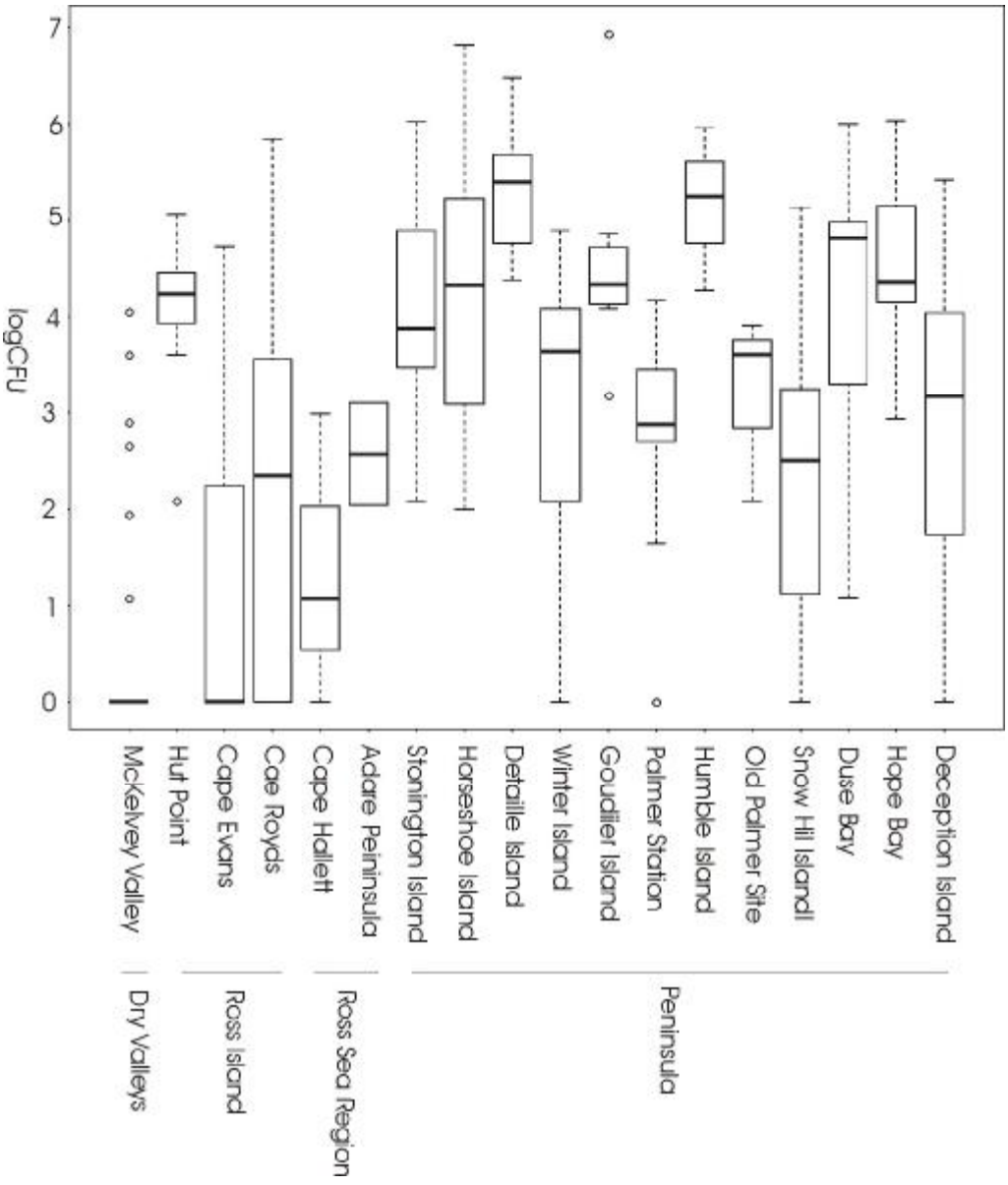

Figure 3.3. Boxplot of log transformations of fungal CFUs from soil samples from the 18 locations included in this study.

Table 3.3. Simple regression analysis of soil characteristics of all sample locations using logCFU (fungal CFU + 1) as the dependent variable.

Simple Regression with logCFU

Variable	Regression Trend	R^2	P-value	Equation
pH	-	0.226	<0.001	y= -0.675 + 7.41
Moisture (%)	+	0.110	<0.001	y= 0.043+ 1.994
EC (µS)	-	0.050	<0.001	y= -2.35e-04 + 2.735
Total Carbon (%)	+	0.155	<0.001	y=0.094 + 2.215
Nitrogen (%)	+	0.091	<0.001	y= 0.669 + 2.298
C/N	+	0.047	<0.001	y= 0.029 + 2.164

Table 3.4. Comparison of mean soil characteristics between samples which produced fungal CFUs and samples which produced no CFUs. Mean values and 95% confidence intervals are displayed. All 6 variables were significantly different between the 2 groups ($p<0.05$).

Variable	Fungi Present (169)	No Fungi Present (76)
pH	6.812 ± .218	8.106 ± 0.213
Moisture (%)	14.705 ± 2.69	6.855 ± 1.88
EC (µS)	545 ± 137	2522 ± 1631
Carbon (%)	5.01 ± 1.76	0.225 ± 0.115
Nitrogen (%)	0.448 ± .164	0.057 ± 0.039
C/N	14.3 ± 2.735	7.9 ± 1.678

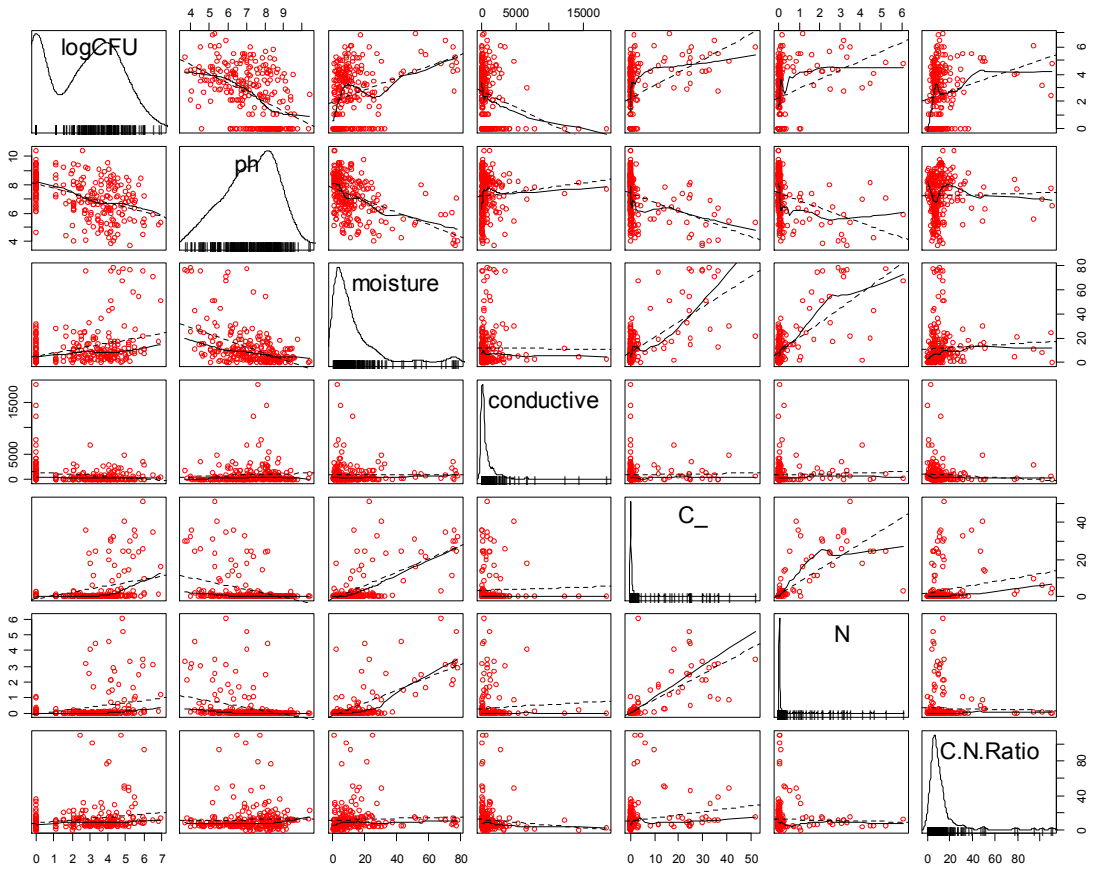

Figure 3.4. Scatter plot matrix of all soil variables. Only CFU has been log transformed. The characteristics of moisture, conductivity, percent C, percent N and C:N ratio are substantially right skewed.

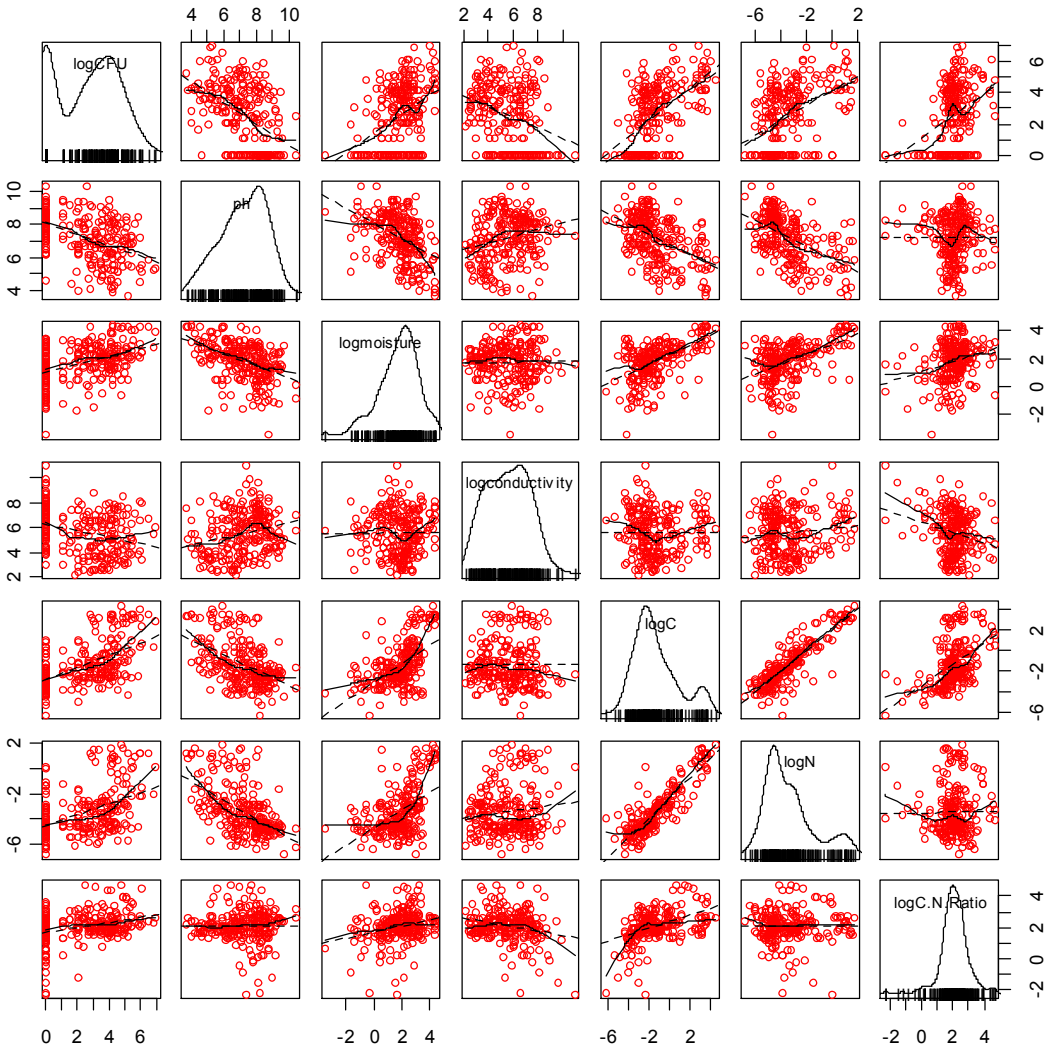

Figure 3.5. Scatter plot matrix of all soil variables. In addition to CFU being log transformed, moisture, conductivity, percent carbon, percent nitrogen, and C/N ratio have been log transformed to reduce the right skewed nature of the data from Figure 3.4.

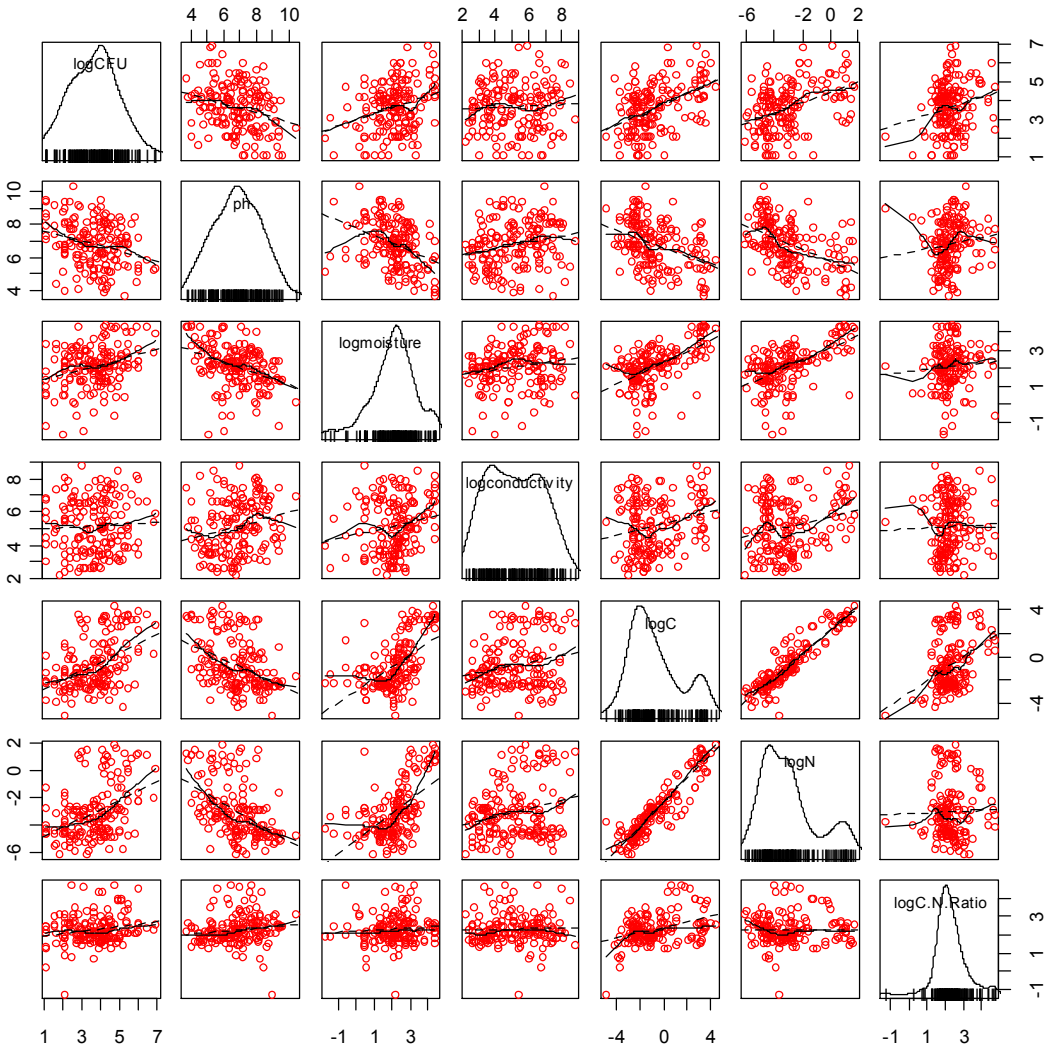

Figure 3.6. Scatterplot matrix of all soil log transformed variables. Samples with 0 CFUs have also been removed so that logCFU will have a normal distribution.

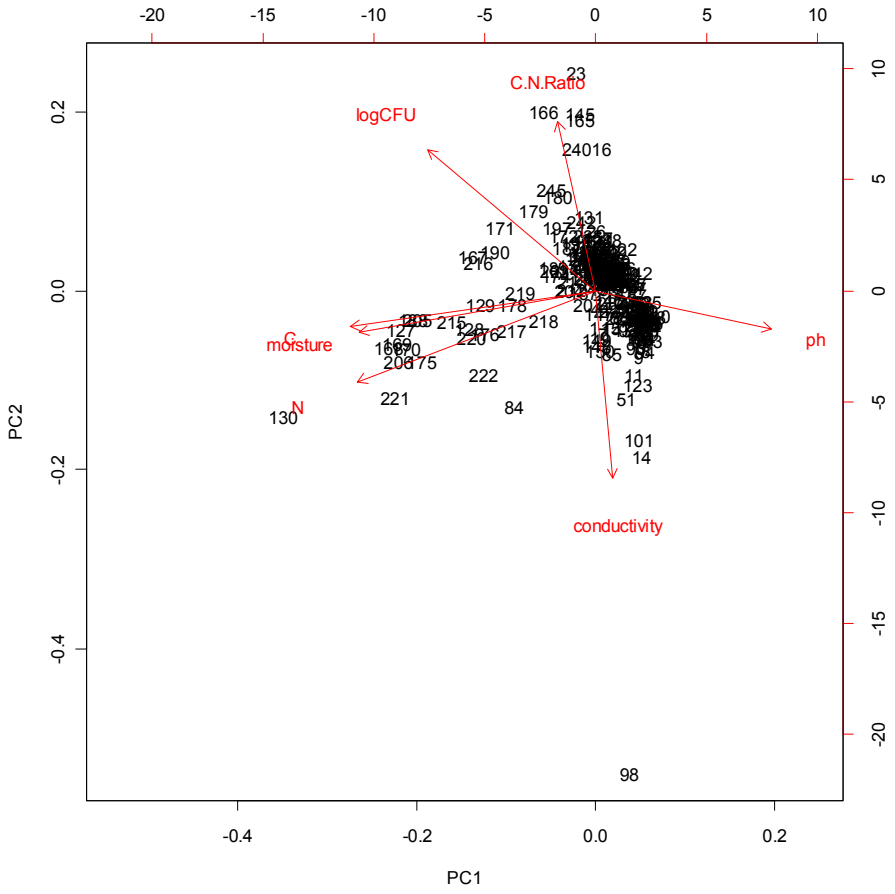

Figure 3.7. Principal Components Analysis of the overall dataset, with 43% of variation accounted for by PC1 and 17% by PC2.

Chapter 4

East Base, SOS: Assessment of deterioration and recommendations for conserving this important Antarctic historic site

Published as

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East Base is located on Stonington Island just west of the Antarctic Peninsula (68°11'S, 67°00'W). It was built in 1940 and is the oldest standing United States base in Antarctica. Over the past decades, the wooden structures at the site have experienced serious deterioration. An assessment of the current condition of the historic structures was carried out in January 2007 as part of a cooperative expedition by the National Science Foundation and the British Antarctic Survey. Many forms of abiotic deterioration and biotic degradation are affecting the wooden buildings and artifacts. Wind is eroding the exterior wood surfaces and salt accumulations in wood cause a corrosion and defibration. Wood and other organic materials in contact with the ground and where moisture accumulates are also being attacked by wood-destroying fungi. Previous conservation work completed in 1992 at the site has helped to partially protect the buildings but deterioration since that time has increased. These historic structures urgently require conservation work to insure their preservation. Attention is desperately needed as soon as possible or the structures and artifacts at this important historic monument will be lost.

History of Site

East Base was constructed in 1940 by the US Antarctic Service expedition led by Admiral Richard Byrd. Stonington Island was chosen because of its unique southerly location along the Antarctic Peninsula and a glacial ramp connected the island to the

Peninsula, providing easy access for dog sledding teams to reach the mainland throughout the year. The base was occupied until March 1941, and later used by the British in 1946 as a temporary shelter while they were constructing their own base on the Island, designated as Base E. East Base was again occupied by the American Ronne Antarctic Research Expedition (RARE) in 1947 led by Finn Ronne. This expedition remained at the site until 1948. During this time great advances were made in mapping of the previously unexplored area of the peninsula south of Stonington. This expedition marked the first overwintering by a woman in Antarctica. After 1948, it was never again utilized by an American team but was used by the British at Base E until they abandoned Stonington Island in 1975 because the glacial ramp had deteriorated and access to the mainland was eliminated. An account of the history at East Base and condition of the buildings at the time was made by Lipps (1978).

Past conservation

East base is the oldest existing United States Antarctic base and was designated Historic Monument #55 by the Antarctic treaty nations in 1989. It was visited by personnel from the National Park service in 1991, and an East Base Management Plan was developed (Spude and Spude, 1993). This plan outlined several actions needing immediate attention and listed many long term recommendations. In 1992, some of the recommended repairs were made and the condition of the historic site further documented (Parfit, 1993). A more detailed account was also published on the project in 2002 (Broadbent and Rose, 2002). Since 1992, no other work has been done at the site and this

paper provides an in depth account of the deterioration taking place and documents the current condition of East Base.

2007 Assessment and Current Condition of East Base

There are 3 buildings currently standing at East Base, the Main Building (former bunkhouse), Science Building and Ronne Hut (Fig. 4.1). In addition, the floor timbers of two other buildings, the Machine Shop and a storage shed, are present. The original construction of the buildings was with 8 by 4 foot prefabricated panels with a plywood interior and a drop siding exterior. The 3 ½ inch gap between the interior and exterior walls was filled with rock wool insulation. After construction, the buildings were covered with a layer of canvas to prevent wind and snow from entering the structures through cracks and crevices. Little of this canvas layer remains except at some sections on the main building that are leeward from the prevailing winds and partially covered by a large snow bank. Portions of each of the buildings were later covered with black "Ruberoid" felting material by British personnel at Base E. Some of this material still remains but most has been stripped away by the strong winds that occur at the site.

The British made substantial modifications to all three buildings and their original uses would not be readily apparent to a visitor without interpretive materials. The Science Building was used as a sledge workshop and for tent storage. The Ronne Hut was used as an emergency generator site and a concrete slab has been placed in a hole cut into the floor. The Main Building received the greatest modification of all three buildings as the original floor was apparently covered with a thick layer of ice when the British returned

to Stonington in 1957 so they built another floor four feet above the original. A partitioning wall was constructed on the eastern side of the building which houses a room with the original floor. A large portion of the main building was used for storage and for seal processing as seal meat was used to feed the dogs on the sledging teams. Consequently the interior of the main building is filled with seal carcasses in addition to ice and other debris. The British constructed floor is also collapsing in many areas making the interior of this building hazardous to enter (Fig. 4.2). There is a broken door on the north side of the building, and an entrance in the roof on the south side which provides entry via a ramp to the collapsing floor. The door on the eastern side of the building leading to the room with the original floor has broken off in the last 15 years apparently due to corroded hinges. There is also a large number of missing roof timbers and wall slats on the building with many more timbers missing on the main building then the two other buildings (Fig. 4.3).

The Science Building and Ronne Hut are in relatively good condition compared to the Main Building. Because they were comparably weather tight, the conservators in 1992 chose the Science Building to set up a museum type display of artifacts that had been found outside (Fig. 4.4). These artifacts were from both the 1941 USAS and 1947 RARE Expeditions. Additionally, there are some materials, such as sledging flags and dog harnesses from the time after 1948 when the British occupied the structures. A cache was also made in the Ronne Hut for additional artifacts that the 1992 conservation crew thought deserved additional protection.

In addition to the wooden structures, there are numerous dump sites and caches located around the buildings. These sites were mapped extensively and reported in the East Base Management Plan (Spude and Spude, 1993). Most of these materials were covered by a light layer of gravel by the 1992 team and they remain buried. No excavations were done and an assessment of their condition was not made. One dump site contains a World War I era light tank and an artillery tractor (Fig. 4.5). These vehicles were brought to the island by the USAS team in 1940 but proved to be of little use because of inadequate traction in the deep snow. Another object of historic interest is an aircraft engine still in its packing crate near the science building.

The cold temperatures of the polar environment have provided some protection from microbial decay, as compared to what may occur in temperate or tropical areas and the isolated location of Stonington Island has saved artifacts from pilfering. However, significant and serious deterioration to the historic buildings and artifacts are being experienced. Losses are occurring from environmental (wind) damage, salt defibration and biodegradation of wood due to fungal decay.

Exceedingly strong winds at the site can be very damaging. This was experienced during the site visit when winds of approximately 80 knots from the North East occurred for most of the day. Although this made outside assessment work virtually impossible, it did allow for valuable observations to be made on the effect of wind on the structures. With the eastern door having previously collapsed on the main building, wind was funneled into the room and pushed upward on the roof. On the southeast corner of the building, the roof was observed to rise up from the wall repeatedly throughout the day by

about 15 cm. During this time, one roof board was observed to fly off the science building roof. This demonstrates the seriousness of the current situation and potential for the roof and walls of the building to be completely destroyed if conservation efforts are not initiated soon.

There is also evidence of surface erosion of wood boards due to the wind but it appears to be limited because of the lack of small soil particles on Stonington Island. The heavy erosion of wood at the historic huts built by Scott and Shackleton on Ross Island seems to be exacerbated by the loose volcanic scoria on the ground that causes a sand blasting effect when winds are strong. This small loose volcanic soil is not present at Stonington Island and the wood erosion observed here is probably primarily due to ice crystals blasting the wood during storms.

Salt corrosion of wood and surface defibration was also apparent on some areas of the wooden structures. This was most obvious on the interior walls of the meteorological tower attached to the science building. The process of salt corrosion of wood in polar environments has been previously described by Blanchette et al. (2002). Absorption of sea spray on wood and evaporation results in high concentrations of salts on wood surfaces. The high salt concentrations cause a chemical attack and a breakdown of the middle lamella that attaches wood cells to one another. This leads to a defibrated "fuzzy" appearance on wood surfaces and detached wood cells that can easily become removed by the wind. Periodic rainfall occurring on Stonington Island can leach the salts from the wood and may be limiting the accumulation on exterior surfaces. In areas protected from rainfall, such as the windward facing walls inside the science building where salt spray

gets blown into the building, have significant defibration. The collapsed door on the northern side of the met tower is also an entrance facilitating entry of salt spray into the building interior and the salt defibration is most notable at this location.

Another serious concern is degradation by wood destroying fungi. Although wood decay may be considered unusual and unexpected in Antarctica, it occurs frequently on wood that has been introduced to the continent. Decay of historic woods in Antarctica was reported by Blanchette et al. (2004) occurring at the Ross Island historic expedition huts. Although the polar environment is exceedingly cold and dry, environmental monitoring within the Ross Island huts revealed that there was appreciable amounts of time in the Austral summer when temperature are above 0º Celsius and relative humidity above 80% (Held et al., 2004) providing time periods that are conducive for fungal growth. In addition to concerns about the soft rot type decay that occurs in the wood, other fungi, such as surface molds cause dark discoloration on wood and other organic based artifacts. Evidence on the diversity and distribution of these species indicates that most of the species responsible for degradation are indigenous to Antarctica and therefore well adapted to the rigors and extremes of the Antarctic environment.

Our preliminary investigations at East Base indicate that soft rot fungi are present and are causing significant wood decay. Dark staining surface molds that disfigure wood are also present and causing problems. Taxonomic studies reveal that some of the same wood destroying fungi responsible for the soft rot in the Ross Island huts, specifically fungi in the genus *Cadophora*, are also present on wood at East Base. The actual amount

of wood decay in the various buildings and wooden artifacts at East Base was not able to be determined during this preliminary assessment. The amount of decay present in woods that are in contact with the ground needs further investigation to determine the extent of colonization and loss of structural integrity that has occurred.

The fungi most abundant as surface molds found inside the structures at East Base, specifically *Cladosporium* and *Geomyces,* also have been found at the Ross Island huts (Arenz et al., 2006). Interestingly, East Base is located nearly 10 degrees latitude further north than the Cape Evans hut on Ross Island and has a generally warmer and more humid environment but our studies show fewer active molds inside the buildings at East Base. Environmental monitors were left at East Base in January 2007 and will be used to review precise environmental conditions at the site. Possibly, greater air circulation and drier conditions occur in these generally open buildings at East Base and this discourages mold growth.

Most of the windows and skylights in the building, having been broken or missing, were reinforced with Plexiglas by the 1992 conservation team. This seems to have been an effective repair as in only one skylight was an interior Plexiglas pane found to have subsequently fallen out, and no examples of broken panes were found. However, an attempt to protect the 1941 visitor welcoming message, left by then base commander Richard Black in the science building, seems to have had the opposite effect as it is now completely illegible. A Plexiglas pane was placed directly against the wall to cover the message and this apparently trapped moisture behind it resulting in extensive fungal growth. A similar effect was found on two pages from Jennie Darlington's book "My

Antarctic Honeymoon" (Darlington and McIlvaine, 1956) fixed to the wall in the partitioned smaller room in the main building. The pages were covered with mold and are becoming illegible due to the dark fungal discoloration caused by the Plexiglas trapping moisture which encourages their growth. Other interpretive panels placed in the science building that were completely enclosed from all sides are in good condition by comparison.

Recommendations

The deterioration occurring at East Base is significant and repairs, conservation and additional investigations are needed as soon as possible. All open or missing doors and windows in the three buildings should be repaired or replaced to preserve the remaining integrity of the structures. In the Main Building, all three entrances need to be closed to the outside environment; the broken door on the north side of the building, the open ramp entrance on the roof and the door on the eastern side. As a temporary repair, the eastern side door from the inside of the building was nailed shut during our visit but a more permanent repair is needed. If regular access for visitors to this room is desired, a door with non-corrodible hinges should be used. The Ronne Hut door is still in place but open and only a snow drift presently inside the room prevents the door from being completely opened. If this is to be a secure site for artifacts, the door must be secured. Repairing these openings will help protect the structures from the very high winds which occur here as well as helping to prevent some of the snow drifting inside, which when it melts will provide sufficient moisture needed for fungal growth to occur.

Significant work will need to be done on the roofs of all three structures to make them relatively weather tight. One particular weak spot is the roof on the south-east corner of the main building which moves up and down during windy days and the roof may completely detach soon. Hopefully, repairs to the door made in January 2007 will help prevent wind from pushing the roof up from underneath and will at least allow it to remain attached. However, the roof must be reattached to the wall and reinforced for longer term structural stability.

The original conservation objectives for the buildings and artifacts outlined in the 1993 Conservation plan written by the National Park Service (Spude and Spude, 1993) are for long term preservation efforts and many aspects such as the biotic and abiotic degradation taking place are not addressed. If these historic buildings and artifacts are to be saved, an interdisciplinary team, such as that organized by the Antarctic Heritage Trust for preservation of the Ross Sea huts, should be established. Some immediate recommendations, however, can be made. Moisture must be excluded from the interior hut environment as this is supporting fungal growth and serious deterioration of the wood and artifacts. In addition to repairing the doors, work needs to be done to the roofs of all three buildings to make them reasonably water tight. If current environmental trends in the area continue, leading to warmer and wetter conditions, this will become even more essential. If left undone the existing decay fungi will progressively cause more damage and losses.

Although Stonington Island is relatively remote, it does receive visits by cruise ships, and over 600 signatures were counted on the guestbook that had been left by the conservators in 1992. Given current trends of increasing Antarctic tourism, this very significant historic site will undoubtedly have even more visitors in the future. Considering that parts of the huts could represent safety issues, particularly the false floor in the main building, work is needed urgently to deal with these concerns.

The presence of the United Kingdom's Base E just a few hundred meters away from the U. S. East Base and the historic use of Base E by the British after 1941 strongly suggests any conservation efforts should be coordinated with the British Antarctic Survey. Both bases need conservation for successful preservation in the future. The success attained at East Base in exploring the geography of the Antarctic Peninsula would not have been possible without collaboration between the US and the UK, and the history of both sets of buildings is tied to the other. Collaborative efforts for conservation of both Bases are strongly encouraged to insure these important historic Antarctic sites are protected for future generations.

The site represents a remarkable period of history when the last of the empty spaces on the Antarctic map were being filled in, and rightly deserves it designation as a historic monument. The conservation work done at this site in 1991 and 1992 was commendable, especially considering the short amount of time the team stayed at the site. However, the buildings at East Base are now at a critical stage. They are currently standing but if current deterioration is allowed to continue their decline will not be a gradual one. The high winds in this area expose even small structural weaknesses to

potential losses of building parts. The detachment of roof or wall boards will undoubtedly lead to rapid destruction and collapse of the entire structure. Michael Morrison, an architect from the United Kingdom who was a part of the BAS heritage survey team remarked that "I do think that a joint agreement over the management and future of the site is urgently needed between the United Kingdom and the United States. I feel that unless something is done urgently the situation will change from being a problem into being a disaster!". Conservation work and continued investigations on the agents causing deterioration must be completed at this site soon or the opportunity for such work will be gone and this incredible monument to Antarctic exploration will be lost.

Figure 4.2. East Base located on Stonington Island, Antarctica showing the Ronne hut (left), Main Building (center) and Science Building with Met Tower (right) as they appeared during the assessment in January 2007.

Figure 4.2. The Main Building showing collapsing floor built by British Antarctic personnel from Base E. This floor was built 4 feet above the original floor and covers historic artifacts, debris and ice.

Figure 4.3. Exterior north wall of the Main Building showing the loss of many wall boards.

Figure 4.4. Inside the Science Building showing the artifact display set up by 1992 Conservation team.

Figure 4.5. Stonington Island has many large artifacts remaining from the 1941 expedition including a World War I era tank and tractor (in background).

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