LETTERS
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Relationship between soil fungal diversity and temperature in the maritime Antarctic

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Soil fungi have pivotal ecological roles as decomposers, pathogens and symbionts[1,2]. Alterations to their diversity arising from climate change could have substantial effects on ecosystems, particularly those undergoing rapid warming that contain few species[3,4]. Here, we report a study using pyrosequencing to assess fungal diversity in 29 soils sampled from a 1,650 km climatic gradient through the maritime Antarctic, the most rapidly warming region in the Southern Hemisphere[5,6]. Using a 'space-for-time' substitution approach, we show that soil fungal diversity is higher in warmer habitats, with increases of 4.7 (observed) and 11.3 (predicted) fungal taxa per degree Celsius rise in surface temperature along the transect. Among 22 predictor variables, air temperature was the strongest and most consistent predictor of diversity. We propose that the current rapid warming in the maritime Antarctic (0.34 °C per decade[6]) will facilitate the colonization of soil by a wider diversity of fungi than at present, with data from regression models suggesting 20–27% increases in fungal species richness in the southernmost soils by 2100. Such increases in diversity, which provide a sentinel for changes at lower latitudes, are likely to have substantial effects on nutrient cycling and, ultimately, productivity in the species-poor soils of maritime Antarctica.

The maritime Antarctic is undergoing rapid climate change. Surface air temperatures in the region, which broadly encompasses the Antarctic Peninsula and islands of the Scotia Arc, have risen by up to 2.8 °C over the past 50 years, at rates several times that of the global mean[5,6]. Rising temperatures in the region have led to changes to the physical environment, including ice shelf collapses and glacial retreats[5]. However, in recent years, biological responses to warming have also become apparent across the region[7]. These include order of magnitude increases in the population sizes of the two native angiosperms, increased moss growth rates, and the establishment of non-native plant species[8–10]. The range expansions of native plant populations and the establishment of non-native species in the typically unvegetated soils of the region are thought to be associated with new areas of land becoming exposed following glacial retreat, enhanced plant growth and reproduction, and accelerated soil nutrient cycling[7,10,11].

Although climate change effects on the maritime Antarctic flora have recently become apparent, far less is known of soil microbial responses to warming in the region. Artificial warming experiments in the natural environment have shown relatively minor changes to the composition of bacterial communities in response to increased soil temperatures (0.5–2 °C annual means), which is not surprising, as the experiments have only lasted for one to three years[12,13]. However, the responses of soil fungi to climate warming in the maritime Antarctic have yet to receive attention. Despite their pivotal importance in terrestrial ecosystems as decomposers, pathogens and symbionts[1,2], the majority of fungi are filamentous in form and—especially for the lichens—grow slowly in the natural environment[14], hampering assessments of their responses to warming treatments. For instance, in the low Arctic, substantial changes to root symbiotic fungal communities in response to warming only become apparent after 17 years of treatment[15]. Here, to circumvent the problem of detecting the responses of these slow-growing microbes to warming manipulations, we studied fungi in soil sampled from along a natural climatic gradient through the maritime Antarctic. Using a similar approach to previous 'space-for-time' substitution studies[16,17], we employ the gradient as a proxy to predict changes to soil fungal communities arising from climate warming in the region. We show that surface air temperature is a significant factor shaping the diversity and composition of soil fungal communities. On the basis of our observations, we predict that future warming in the region will lead to 20–27% increases in the numbers of fungal species present in the southernmost soils of the region by the end of the century, and that this will have consequent effects on biological productivity.

We studied 29 soils sampled during the 2007–2008 austral spring and summer from along a 1,650 km gradient between 72° S and 60° S (Fig. 1 and Supplementary Table 1). The soils were free of vegetation (Supplementary Fig. 1), and were hence representative of the barren soils that are frequent in maritime Antarctic terrestrial ecosystems. Data from the Regional Atmospheric Climate Model[18] indicated a significant increase in mean annual surface air temperature (MASAT) between south-eastern Alexander Island (72° S; MASAT −11 °C) and Signy Island in the South Orkney Islands (60° S; MASAT −4 °C), with a 0.62 °C increase in air temperature for each degree decrease in latitude (Fig. 1, upper inset; Supplementary Table 2). To determine whether other abiotic parameters varied along the transect, we analysed soils for a suite of 20 physicochemical parameters (including pH, electrical conductivity and the concentrations of 11 elements and five dissolved ions). Soil C:N ratio declined significantly at lower latitudes ($R^2 = 33\%$, $P = 0.001$; Fig. 1, lower inset; Supplementary Table 2) and was negatively associated with MASAT ($R^2 = 31\%$, $P = 0.002$). This is consistent with previous observations that soils in cold ecosystems have higher C:N ratios than those in warmer

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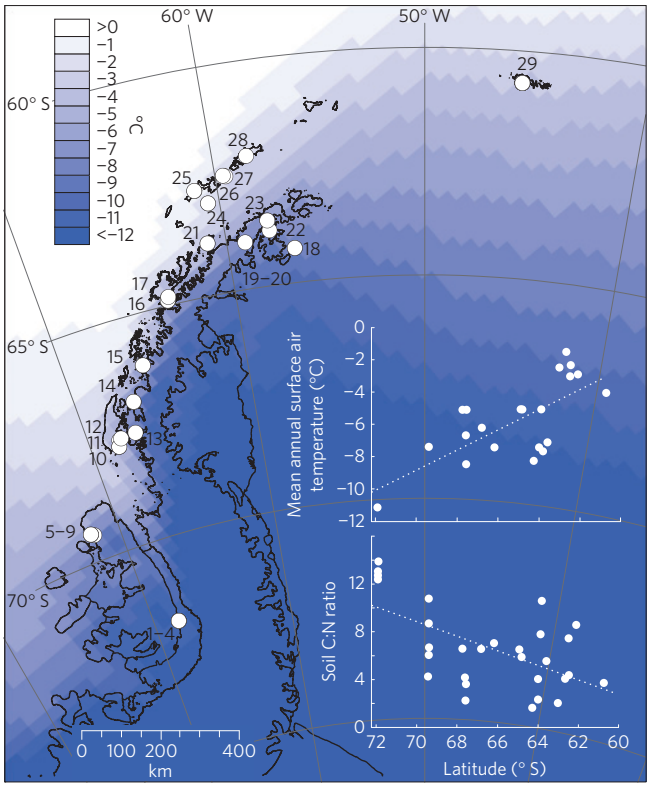

Figure 1 | Locations of sampling sites along the climatic gradient. Site names, latitudes, longitudes and altitudes are shown in Supplementary Table 1. Upper inset shows mean annual surface air temperature at each sampling location for 2007, derived from the Regional Atmospheric Climate Model[18], as a function of latitude. Lower inset shows soil C:N ratio as a function of latitude.

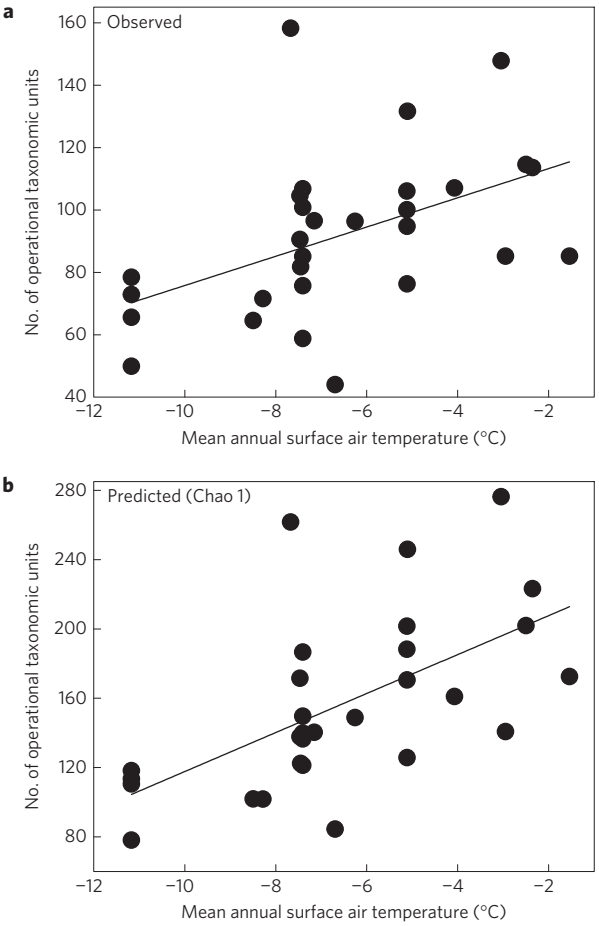

Figure 2 | The influence of mean annual surface air temperature on the numbers of soil fungal taxa along the climatic gradient. a,**b**, Data shown are the numbers of observed (**a**) and predicted (**b**) fungal taxa recorded in the 29 soils as a function of mean annual surface air temperature. Taxa were grouped at 97% similarity. The numbers of observed and predicted operational taxonomic units in each soil are shown in Supplementary Table 1.

ecosystems[19], most likely because of slow organic matter decay[1]. The strong influence of latitude on MASAT, and its weaker effect on soil C:N ratio, were also confirmed by structural equation modelling (Supplementary Fig. 2). None of the other parameters that we measured, including the altitude from which the samples were taken, varied significantly with latitude (Supplementary Table 2).

We measured the numbers of fungal species present in soil along the climatic gradient by PCR amplifying and pyrosequencing internal transcribed spacer 2 regions of fungal ribosomal RNA operons. Each fungal taxon was represented by an operational taxonomic unit (OTU), which differed by >3% in sequence identity from other OTUs. In contrast with studies on other continents, where woody plant species are present and ectomycorrhiza-forming basidiomycetes are consequently frequent in soil[2], we found relatively few basidiomycetes, with ascomycetes dominating the soil fungal communities (Supplementary Fig. 3). When stepwise multiple regression analyses were used to assess how the numbers of observed and predicted (Chao 1) OTUs responded to the 22 predictors along the gradient, MASAT was consistently the best predictor for both measures of soil fungal diversity (Table 1), explaining 23% and 39% of variation in the numbers of observed and predicted OTUs, respectively. Univariate regression analyses indicated significant positive associations between MASAT and the numbers of fungal OTUs, with mean increases of 4.7 observed OTUs and 11.3 predicted OTUs per degree Celsius rise in air temperature along the transect (Fig. 2a,b). Soil potassium (K) concentration, which is known to be positively associated with the frequencies of fungi[20], was also included in stepwise regression

Table 1 | Data from stepwise multiple regression models using observed and predicted (Chao 1) numbers of OTUs as response variables.

Response variable	R^2 (%)	Predictor variables	Slope	*F* value	*P* value
Observed no. OTUs	35	MASAT*	4.7	9.15	0.006
		Soil K concentration	4.4×10^{-3}	4.83	0.037
Predicted no. OTUs	57	MASAT*	14.8	24.55	<0.001
		Soil K concentration	9.2×10^{-3}	8.12	0.009
		Soil C:N ratio	4.8	4.46	0.045

*Mean annual surface air temperature. Error degrees of freedom in all analyses were 26. MASAT and K concentration were expressed in degrees Celsius and mg kg^{-1} in these analyses, respectively, and C:N ratio was dimensionless. Intercepts in multiple regression models for observed and predicted numbers of OTUs were 112 and 199, respectively.

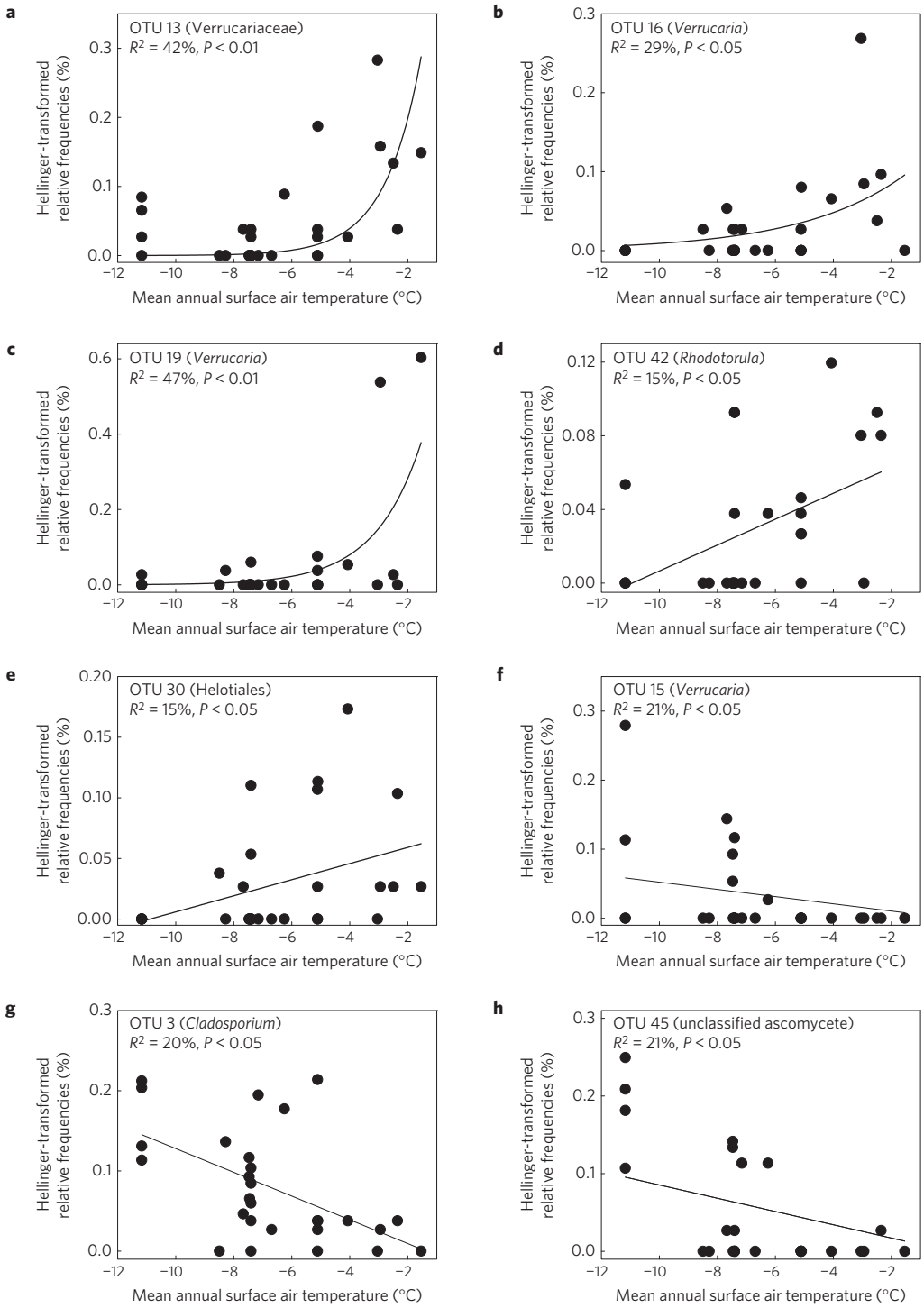

Figure 3 | The influence of mean annual surface air temperature on the frequencies of eight fungal taxa. a–h, Lines of best fit are either linear or polynomial functions. Note that *x* axes are all identically scaled.

models, and explained 12–14% of variation in the numbers of OTUs (Table 1). C:N ratio, also previously shown to influence soil fungal abundances[2,20], explained a small (4.3%) but significant amount of variation in the predicted number of OTUs (Table 1).

Redundancy analysis (RDA) was then used to determine how the frequencies of the fungal OTUs varied with the 22 predictors across the gradient. These analyses indicated that changes in soil fungal community composition were best predicted by MASAT ($R^2 = 6.1\%$, $P < 0.001$), with soil pH, manganese (Mn) concentration and C:N ratio predicting significant, but smaller, amounts of variation in composition ($R^2 = 4.8\%–3.8\%$, all $P < 0.03$). MASAT was the best predictor for three OTUs closely matching mycobionts of the lichen genus *Verrucaria* or the family Verrucariaceae (Supplementary Fig. 3), which each increased in frequency in warmer soils (Fig. 3a–c). *Verrucaria* is typically a rock-inhabiting genus in the Antarctic[21] and it is hence likely that we detected the fungus in its free-living state (or as spores or other propagules) in soil. RDA based on presence–absence data indicated that changes to the frequencies of *Verrucaria* largely accounted for the increase in fungal diversity in warmer soils, corroborating the previous

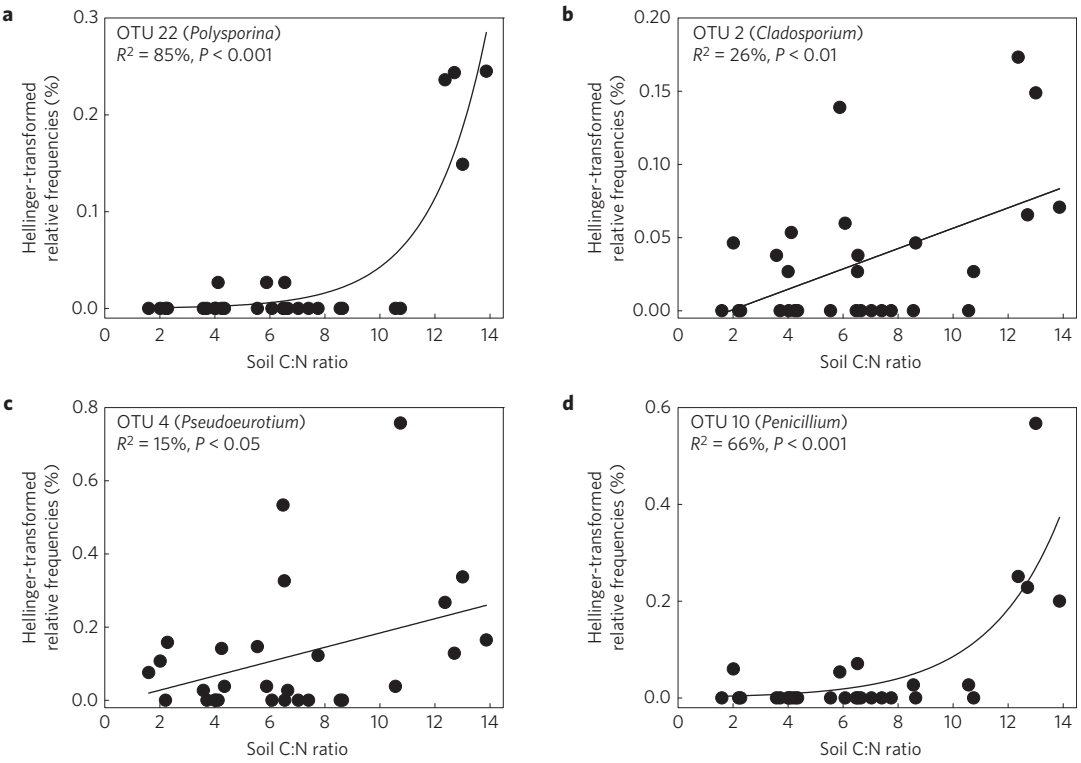

Figure 4 | The influence of soil C:N ratio on the frequencies of four fungal taxa. a–d, Lines of best fit are either linear or polynomial functions. Note that *x* axes are all identically scaled.

observation that the numbers of maritime Antarctic lichen taxa increase sharply between 72° S and 60° S (ref. 22). The yeast *Rhodotorula* and a member of the Helotiales, representatives of which are known to be present in Antarctica (Supplementary Table 3)[23], also accounted for increases in fungal diversity in warmer soils (Fig. 3d,e). In contrast, we found that the frequencies of three other OTUs were best predicted by MASAT (Supplementary Fig. 3), but that these fungi, which matched closely with a *Verrucaria*, *Cladosporium* and an unclassified ascomycete, decreased in frequency in warmer soils (Fig. 3f–h). As C:N ratio was associated with air temperature (Fig. 1, lower inset), and there was a priori evidence indicating that this parameter is associated with climate[1,19], we also included the ratio in the RDA analyses. Soil C:N ratio was found to be positively associated with the abundances of OTUs matching closely with the lichen-forming ascomycete *Polysporina* and the free-living ascomycetes *Cladosporium*, *Pseudoeurotium* and *Penicillium* (Fig. 4a–d and Supplementary Fig. 3), confirming the direct influence of this parameter on soil fungal community composition[2,20]. Associations between OTU frequencies and soil pH, K and Mn concentrations, which influenced soil fungal diversity but did not alter along the gradient, are summarized in Supplementary Fig. 3.

Our observations show that surface air temperature is an important factor shaping the diversity and composition of maritime Antarctic soil fungal communities. Because liquid water availability is tightly coupled with air temperature in maritime Antarctic environments[24], it is likely that part of the observed pattern of increasing fungal diversity in warmer habitats is due to improved access to water, which, in combination with higher temperatures, will enhance metabolic activity, extend the period for which fungi are active each year, and enable a switch from survival to growth and dispersal strategies. Our observations thus extend into the Antarctic the recent finding that climate has an important influence on soil fungal diversity on the Earth's other six continents[2]. They suggest that declines in soil C:N ratio arising from climate warming in the maritime Antarctic might result in reductions in the frequencies of several ascomycetes in soil, including the widespread saprotrophs *Penicillium* and *Cladosporium*, which are known to be of importance in soil decomposition processes[1]. However, the overriding impact of warming is likely to be an increase in the number of fungal taxa present in the soils of the region. The data here indicate that increases are likely in the frequencies of mycobionts of the lichen-forming genus *Verrucaria*, which are known to influence rock weathering processes, the yeast genus *Rhodotorula*, and the order Helotiales, some members of which positively affect the *in vitro* growth of the angiosperms spreading throughout the region[25].

The rate at which fungal diversity will increase in maritime Antarctic soils will be influenced not only by rising air temperatures but also by precipitation, which is likely to increase as the region warms[6], and by vectors such as air currents and human activities, including tourism[9]. However, assuming a current rate of warming over land in the maritime Antarctic of 0.34 °C per decade[6], and increases of 4.7 observed species of soil fungi per degree Celsius rise in air temperature, our analyses (Table 1) suggest up to a 20% increase in the numbers of fungal species present in the southernmost soils of the region by 2100. The estimates for the predicted numbers of fungal species are similar, with multiple regression models—taking into account both increases in air temperature and decreases in soil C:N ratio (Table 1)—indicating a 27% rise in the numbers of species present in soils at the highest latitudes by the end of the century. Given the pivotal roles of soil fungi as decomposers, symbionts and pathogens[1,2,20,26] and the demonstrable positive effects of increasing soil fungal diversity on productivity in species-poor soils[3,4], our data suggest substantial impacts of such increases on the ecology of maritime Antarctic terrestrial ecosystems, and on other low-diversity soil fungal communities at lower latitudes.

Methods

Methods and any associated references are available in the online version of the paper.

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Author contributions

K.K.N., P.G.D., S.P.R., A.G.O'D. and D.W.H. conceived this study. P.G.D., K.K.N. and D.W.H. collected soil samples, L.C.C. performed DNA extractions and PCRs. P.G.D. processed sequence data and P.G.D. and K.K.N. performed statistical analyses. P.T.F. provided geospatial data derived from the Regional Atmospheric Climate Model. All authors discussed the results and contributed to the preparation of the manuscript.

Additional information

Supplementary information is available in the online version of the paper. Reprints and permissions information is available online at www.nature.com/reprints. Correspondence and requests for materials should be addressed to P.G.D.

Competing financial interests

The authors declare no competing financial interests.

Methods

Soil sampling. To eliminate any effects of the presence of plants on soil fungal diversity, we sampled only soils without plant cover from along the climatic gradient. The uppermost five centimetres of soil was collected in 50 ml DNA/RNAase-treated plastic tubes (30 mm diam.) from each of five locations at each site and was bulked. The soil was then immediately snap-frozen by immersion in a mixture of dry ice and ethanol (*c*. −80 °C). Samples were maintained at −80 °C from the time of sampling until they were processed.

Air temperature data. The inaccessible nature of most of the sites studied precluded the measurement of soil temperature, and so mean annual surface air temperature (MASAT) data, derived from the Regional Atmospheric Climate Model over Antarctica[18], gridded at a horizontal resolution of 55 × 55 km, were used as predictors of soil fungal diversity. MASAT values for the year 2007 in the grids in which each of the sampling sites occurred were used in statistical analyses (see below).

DNA extraction, PCR amplification and 454 pyrosequencing. Total DNA was extracted under sterile conditions from 10 g of soil using a PowerMax Soil DNA isolation kit (MO BIO Laboratories) as per the manufacturer's instructions. The internal transcribed spacer 2 (ITS2) region of the ribosomal RNA encoding genes was amplified by polymerase chain reaction (PCR) using the primers gITS7 (5′-GTGARTCATCGARTCTTTG-3′ (ref. 27)) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′ (ref. 28)), which target sites in the 5.8S gene and ribosomal large subunit, respectively. The gITS7 primer was 5′-labelled with the 454 FLX sequencing primer adaptor B sequence and the ITS4 primer was 5′-labelled with a sample-specific barcode sequence and the 454 FLX sequencing primer adaptor A sequence. PCRs were performed in duplicate 50 µl reactions, each containing 5 ng template DNA, ×1 Phusion High Fidelity PCR Buffer (New England Biolabs), 0.2 mM of each of the dNTPs (Invitrogen), 0.3 µM of the ITS4 primer, 0.5 µM of the gITS7 primer, and 1U of 1× Phusion High Fidelity DNA Polymerase (New England Biolabs). Thermocycling conditions were as follows: 98 °C for 30 s, 35 cycles of 98 °C for 10 s, 56 °C for 30 s, 72 °C for 15 s and a final extension at 72 °C for 7 min. Negative controls, consisting of sterile water in place of template DNA, did not yield amplicons. Amplicons were purified using a Wizard SV Gel and PCR Clean-Up System (Promega), quantified with a Qubit fluorometer with a Quant-iT dsDNA HF assay kit and then 72 ng of each sample was pooled. The pooled sample was purified again using a QIAquick PCR Purification Kit (Qiagen), and then sent to Macrogen for 454 pyrosequencing[29].

Processing of sequence data. Sequences were quality filtered and dereplicated using the QIIME script split_libraries.py with the homopolymer filter deactivated[30]. Homopolymer errors were corrected using Acacia v. 1.48 (ref. 31) and fungal ITS2 sequences were then extracted using ITSx v. 1.0.9 (ref. 32) and checked for chimeras against ITS2 sequences in UNITE v. 6 (ref. 33) using UCHIME v. 3.0.617 (ref. 34). At least 1,435 non-chimeric quality-filtered ITS2 sequences were derived from each soil sample. The sequences were clustered at 97% similarity using UCLUST v. 1.2.22. UNITE v. 6 (ref. 35) taxonomy was assigned to representative OTU sequences using BLAST+ v. 2.2.30. Tables containing the abundances of different OTUs and their taxonomic assignments in each sample were generated and the number of reads was rarefied to 1,400 per sample. The mean number of observed OTUs and the estimated total number of OTUs (Chao 1) were calculated using QIIME.

Soil physicochemistry. Soil pH and electrical conductivity were measured in 1:2.5 and 1:5 soil:water (vol:vol) slurries, respectively. Total nitrogen and organic carbon concentrations were determined using an Exeter Analytical CE440 Elemental Analyzer (EAI) following desiccation at 105 °C and treatment with HCl to remove inorganic carbon. Concentrations of Ca, Cu, Fe, K, Mg, Mn, Ni, P and Zn (mg kg^{-1} dry soil) were determined using inductively coupled plasma mass spectrometry (ICP-MS) following reverse aqua regia digests. Water extractable PO_4^{3-}, SO_4^{2-} and Cl^- concentrations (mg kg^{-1} dry soil) were determined using Dionex ion chromatography. Soil NH_4^+–N, NO_3^-–N/NO_2^-–N and dissolved organic carbon (DOC) concentrations (mg kg^{-1} dry soil) were determined in 1M KCl (1:4 dry soil equivalent to solution ratio) followed by automated flow injection analysis (Skalar Analytical B.V.). The moisture content of each soil sample was determined gravimetrically. All analyses were based on soil passed through a 4 mm sieve.

Statistical analyses. The influence of MASAT and soil physicochemical parameters on changes in the composition of soil fungal communities between sites (beta diversity) was assessed using Redundancy Analysis (RDA) with Monte Carlo permutation tests to assess the significance of the constraints. Parameters were chosen for inclusion in the final RDA model by forward selection based on Akaike's Information Criterion (AIC). When the inclusion of a new parameter led to other terms becoming insignificant ($P > 0.05$), it was dropped, leaving the final minimally adequate model. All models were built for Hellinger-transformed OTU abundances. All analyses were implemented in R. Associations between soil fungal diversity, latitude, MASAT and soil physicochemical parameters were examined using polynomial and multivariate regressions. For the univariate models we adopted a principle of parsimony, accepting the model with the least number of coefficients commensurate with the greatest amount of variation (R^2 value) explained. As some of the covariates were themselves related and causally linked (for example, latitude and MASAT), we hypothesized that each could have both direct effects on the response variables and indirect effects through their direct effects on other predictors. For this reason we used structural equation modelling (SEM) to investigate the direct and indirect effects of latitude, MASAT and soil physicochemical parameters on soil fungal richness. On the basis of the output from our univariate models, we created a full SEM model to test the hypotheses that latitude directly affected MASAT, soil C:N ratio and fungal richness, that MASAT directly affected fungal richness, that soil K concentration directly affected fungal richness and that MASAT directly affected soil C:N ratio. The hypothetical full model was then challenged with the data and goodness of fit assessed using Chi-squared tests and root mean square error of approximation (RMSEA) values. Variables and associated relationships were then removed progressively if they were not significant. The Chi-squared goodness of fit tested the difference between the hypothetical model pathway and the observed data. RMSEA values of <0.05 for the model were considered a good fit to the data. SEM analyses were performed in Amos version 22 (SPSS Software).

Accession numbers. The amplicon sequences associated with this study have been deposited in the NCBI SRA under accession PRJNA282894.

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