



Miocene Climatic Optimum fungal record and plant-based CREST climatic reconstruction from southern McMurdo Sound, Antarctica

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Abstract. Deep-time palynological studies are necessary to evaluate plant and fungal distribution under warmer-than-present scenarios such as those of the Middle Miocene. Previous palynological studies from southern McMurdo Sound, Antarctica (SMS), have provided unique documentation for Neogene environments in the Ross Sea region during a time of pronounced global warming. The present study builds on these studies and provides a new climate reconstruction using the previously published SMS pollen and plant spore data. Additionally, 44 SMS samples were reanalyzed with a focus on the fungal fraction of the section to evaluate the fungal distribution under warmer than present conditions. The probability-based climate reconstruction technique (CREST) was applied to provide a new plant-based representation of regional paleoclimate for this Miocene Climatic Optimum (MCO) locality. CREST reconstructs a paleoclimate that is warmer and significantly wetter than present in SMS during the MCO, with mean annual precipitation reconstructed at 1147 mm yr^{-1} (95 % confidence range: $238\text{--}2611 \text{ mm yr}^{-1}$) and a maximum mean annual temperature of 10.3°C (95 % confidence range: $2.0\text{--}20.2^\circ\text{C}$) for the warmest intervals of the MCO. The CREST reconstruction fits within the Cfb Köppen–Geiger climate class during the MCO of SMS. This new reconstruction agrees with previous reconstructions using various geochemical proxies. The fungal palynological analyses yielded surprising results, with only a single morphotype recovered, in low abundance, with concentrations ranging up to 199 fungi per gram of dried sediment. The taxa present belongs to the Apiosporaceae family and are known to be adapted to a wide range of climate and environmental conditions. As fungi are depauperate members of the SMS MCO palynofloras and because the one morphotype recovered is cosmopolitan, using the fungi record to confirm a narrow Köppen–Geiger climate class is impossible. Overall, the study demonstrates refinement of plant-based paleoclimatic reconstructions and sheds light on the limited presence of fungi during the MCO in Antarctica.

1 Background: the Antarctic Miocene Climatic Optimum record

The Miocene Climatic Optimum (MCO) was a striking climatic event worldwide, but its impact on the Antarctic continent and its massive ice sheets had dramatic implications on many other parts of the world. This is in part because the MCO warming affected an ice-covered continent that is currently covered by ice sheets equivalent to a ~ 55 m sea level rise (Fretwell et al., 2013). The onset of continental-scale glaciation in Antarctica occurred at approximately 34 Ma (Shackleton et al., 1975; DeConto and Pollard, 2003; Stickley et al., 2004; Pollard and DeConto, 2009; DeConto and Pollard, 2016). Palynological records from ~ 34 Ma display a shift from rich and diverse vegetation prior to the Eocene–Oligocene (E/O) transition (Askin, 1992, 2000; Askin and Ricci, 1997; Anderson et al., 2011) to an abrupt decline in terrestrial palynomorph concentrations at or around the E/O transition (Warny and Askin, 2011a; Warny et al., 2019). Studies show there was a sharp decrease in vegetation abundance and diversity in Antarctica after the Oligocene (Raine, 1998; Askin and Raine, 2000; Warny and Askin, 2011b) apart from a brief interval of time during the MCO (Warny et al., 2009). The glacial history of Antarctica during the MCO is starting to be well constrained. Geochemical data from deep-sea proxy records highlight a climate during the MCO that was significantly warmer than modern-day temperatures (Lear et al., 2000; Zachos et al., 2001, 2008). At the start of the Miocene, the large glacial event of the Oligocene–Miocene Transition (OMT) is well documented as a discontinuity in the ANDRILL southern McMurdo Sound (SMS) record, suggesting expansion of the Antarctic Ice Sheet (AIS) into the Ross Sea at that time (Steinthorsdottir et al., 2021). During the Miocene, the East AIS underwent oscillations across the Sabrina Coast continental shelf, with evidence from erosive surfaces and subglacial tunnel valleys that indicates massive melting of the ice sheet surface generating meltwater pulses (Gulick et al., 2017). By the Middle Miocene, localized pockets of limited tundra still existed at least until 13.85 Ma in the McMurdo Dry Valley area (Lewis et al., 2008) and 12.8 Ma in the Antarctic Peninsula (Anderson et al., 2011). There are no records of vegetation in Plio-Pleistocene sediments younger than these dates. The transition from temperate, alpine glaciation to a dynamic, polythermal ice sheet took place during the Middle Miocene (Anderson et al., 2011). During the MCO, temperatures have been reconstructed ~ 7 – 8 °C warmer than modern day, and summer sea surface temperatures (SSTs) likely reached 6–10 °C in the Ross Sea (note that global mean surface temperature reached 18.4 °C) with a brief interval of open-water conditions. CO₂ levels reached at least 600 ppm during this interval (You et al., 2009; Levy et al., 2016; Steinthorsdottir et al., 2021). The MCO interval was not driven by a single event, it instead is comprised of a series of maxima and minima in $\delta^{18}\text{O}$ records, which suggests a complex story of ice sheet

fluctuation and reorganizations of oceanic circulation (Holbourn et al., 2022). This complex climatic optimum was succeeded by long-term climatic deterioration towards icehouse conditions (Flower and Kennett, 1994; Shevenell et al., 2004; Holbourn et al., 2007). The ANDRILL AND-2A record, drilled in McMurdo Sound, Antarctica (Fig. 1), is the focus of the present study. It is the most high-resolution Antarctic stratigraphic record of the MCO time interval (Fig. 2) drilled to date in proximity to the Antarctic coastline.

Previous studies associated with the ANDRILL SMS drilling campaign (AND-2A core) depict a warm, moist Antarctica during the MCO interval, with two major abrupt peaks in the abundance of marine, freshwater, and terrestrial palynomorphs (Warny et al., 2009; Feakins et al., 2012; Griener et al., 2015). An interval within the core from 458.11 to 403.47 m b.s.f. is characterized by a concentration increase in in situ palynomorphs composed mostly of pollen, plant spores, and freshwater algae that is dated from 16.56–16.45 Ma (Warny et al., 2009; Griener et al., 2015). A younger interval, ranging in depth between 311.49 to 289.93 m b.s.f., is also characterized by an increase in pollen, plant spores, freshwater algae, and dinoflagellate cysts; this interval is constrained in age between 15.89 and 14.54 Ma (Griener et al., 2015; Warny et al., 2009). The unusually high concentration of palynomorphs recovered for this site suggests a relatively short period of time during the peak of the MCO in which Antarctica became suddenly much warmer (Warny et al., 2009). These authors noted that the absolute abundance of *Podocarpidites* pollen (podocarp conifer) increased up to 16-fold and that *Nothofagidites* pollen (southern beech) increased more than 60-fold. These were the most abundant plants on land in the sediment source area adjacent to the Ross Sea during the MCO, along with mosses and a variety of ground cover angiosperms. This vegetation composition suggests low tundra on coastal plains and a mosaic of tundra with shrub-like southern beech and low podocarp trees in sheltered areas (Warny et al., 2009). Based on the pollen and spores recovered, these authors suggested that Antarctica's summer temperatures likely reached at least 10 °C around 15.7 Ma. The climate was much warmer, and a substantial increase in moisture delivery to the Antarctic coast also occurred. On the basis of hydrogen isotope data measured from leaf waxes, Feakins et al. (2012) reported that Antarctic coastal precipitation averaged 848 mm yr^{−1} and ranged from 228–1500 mm yr^{−1} during the Middle Miocene. These climate fluctuations, along with the release of fresh meltwater to the Ross Sea region, were probably the result of a poleward shift of the jet stream in the Southern Hemisphere. This shift in the jet stream resulted in warmer water being pushed toward the South Pole, allowing some dinoflagellate species to flourish in ice-free conditions and woody plants to thrive in warmer continental temperatures (Warny et al., 2009). A second Antarctic Middle Miocene marine record in Wilkes Land, which is located at a slightly lower latitude compared to ANDRILL AND-2A, also shows

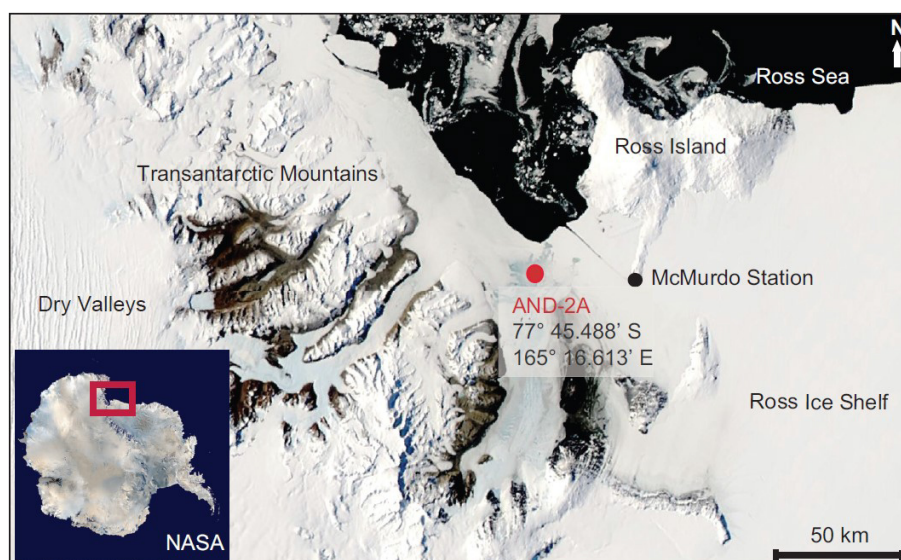


Figure 1. Location of the AND-2A core hole in southern McMurdo Sound, western Ross Sea, Antarctica (Warny et al., 2009).

a dominance of *Podocarpidites* conifers and *Nothofagidites* pollen in an environment of woody sub-Antarctic or sub-alpine vegetation, with some plants representative of shrub tundra and peatlands (Sangiorgi et al., 2018).

Here, we propose revised plant-based temperature and precipitation estimates reconstructed using a climate reconstruction technique (CREST) for the terrestrial source area, and we provide a new fungal record to examine how fungi reacted to the MCO warming.

2 The status of Miocene fungal records

Fungi play key roles in ecosystems; many have restricted geographic distributions, have known host and substrate preferences, and are important local environmental indicators due to their short dispersal distances. Yet, the distribution of terrestrial fungi in marine sediments is very poorly understood, and no data of this type exist for Antarctic sediments deposited during the Miocene Climate Optimum (MCO), between approximately 16.9 and 14.7 Ma. While terrestrial organic matter is relatively scarce in MCO marine sections worldwide (Li et al., 2023), this study of coastal sediments from Antarctica aims to evaluate the presence and type of fossil fungi in addition to plant palynomorphs for interpretation of the expression of terrestrial climate and ecosystem change in areas adjacent to the marine depocenters. Characterizing what the landscape, especially in Antarctica, and climate looked like during the MCO is critical for the modeling of future ecological and climatic warming scenarios; however, excepting a few studies focusing on deeper geologic times, the fungi have been overlooked (Harper et al., 2012, 2016). As the warmest interval of the Neogene Period, the MCO has been identified internationally as an analogue for

the Intergovernmental Panel on Climate Change Representative Concentration Pathway (IPCC-RCP) 4.5–8.5 warming scenarios, both in terms of strength and speed of warming and in terms of latitudinal effects (Intergovernmental Panel on Climate Change (IPCC), 2014, 2021, 2022, 2023). The global synthesis of terrestrial and marine proxies for paleoclimate and paleoecology of the MCO (Steinthorsdottir et al., 2021) did not include the impact of fossil fungal population in their discussion of biodiversity and carbon cycle dynamics simply because of the lack of fungal records. Yet, fungi are vital components of the terrestrial carbon cycle, and their distribution and diversity provide insights into the local ecosystem structure. The study of fungal assemblages and their ecological requirements can also aid in increasing our knowledge of biodiversity changes during rapid climatic shifts (Nuñez Otaño et al., 2021). Fungal remains, including spores and hyphae, are commonly, but not always, preserved as part of the palynological assemblage contained by rocks and sediments (Elsik, 1996; Shumilovskikh et al., 2021). When present in sedimentary sequences, fossil fungi provide a reliable proxy for investigating how fungi respond to warmer global temperatures (Pound et al., 2022). However, studies examining fungal biodiversity in Neogene sediments are historically rare, and it is only recently that focus has shifted to that group (e.g., Rivero de Dibi et al., 2001; Guimarães et al., 2013; O’Keefe, 2017; Quattrocchio et al., 2018; Pound et al., 2019; Romero et al., 2021; Pound et al., 2022). While the majority of fungi are mesophiles (tolerance range 5–35 °C, optima 20–30 °C), a large number of species are able to grow close to or below 0 °C (Dix and Webster, 1995; Robinson, 2001). In order to use fungi for future climatic predictions, we need an in-depth understanding of past fungal community structure and interactions in warmer-than-

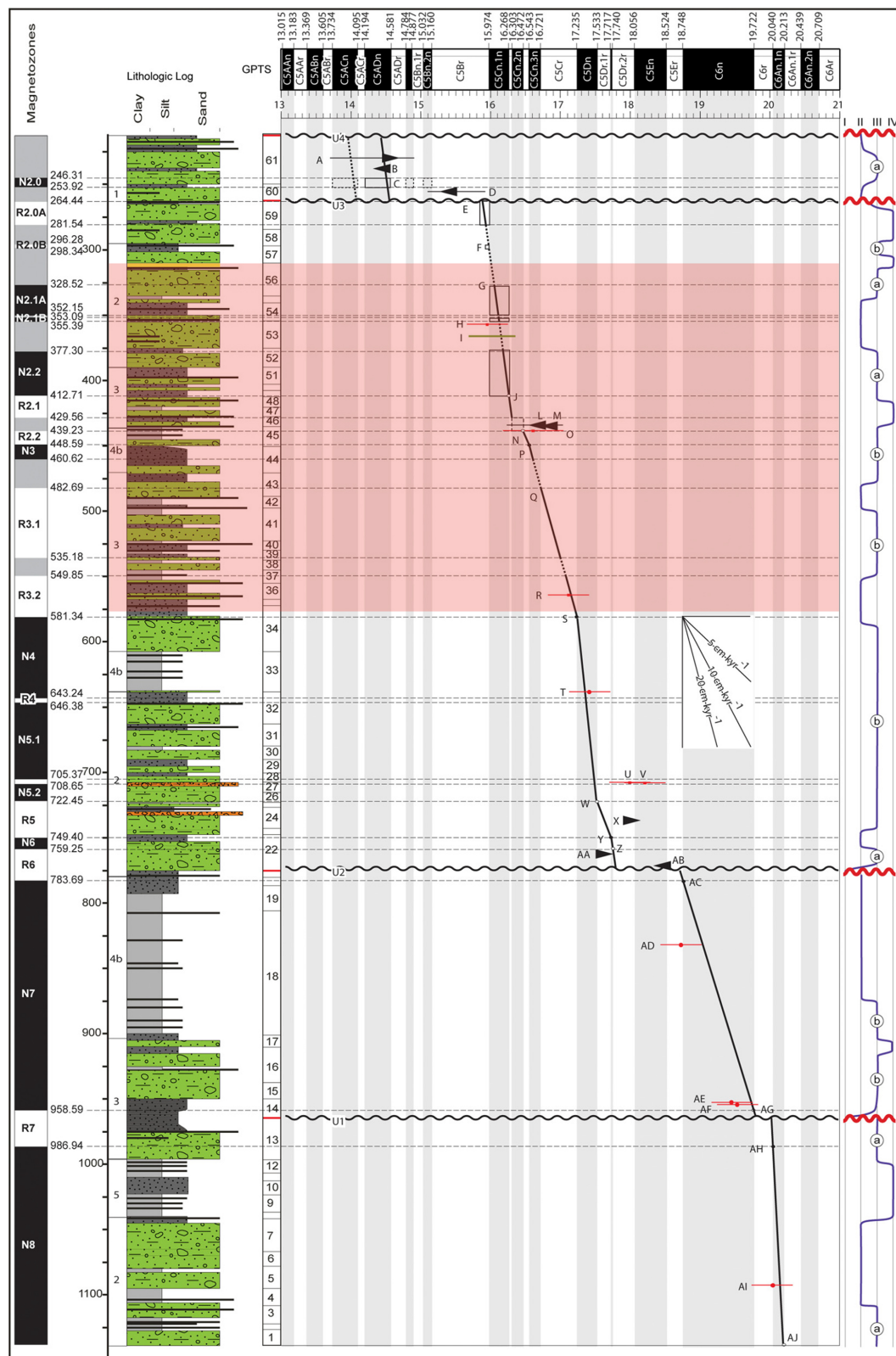


Figure 2. Age–depth model of AND-2A. The highlighted red rectangle signifies the depth of samples studied, covering the Middle Miocene Climatic Optimum. The summary lithologic column is plotted on the far left of the age–depth diagram. The magnetic polarity stratigraphy is plotted to the left of the lithologic column. The black and white bar running along the top of the plot is the geomagnetic polarity timescale with selected chrons labeled (Levy et al., 2016).

present conditions. Without these data, it is difficult to predict future changes in this group and how these will affect ecosystems and, as a consequence carbon cycling, for example. Understanding how climate and ecosystems will adapt to future climate change involves modeling future scenarios by making inferences from geological datasets (Dowsett et al., 2016; Hollis et al., 2019). Paleoclimate research, specifically from the Cenozoic (e.g., Pound et al., 2012; Salzmann et al., 2013; Pound and Salzmann, 2017; Holbourn et al., 2010; Birks, 2019; Warny et al., 2009; Feakins et al., 2012) provides an invaluable source of long-term data that can be used to help understand future climate change. The Fungi in a Warmer World (FiaWW) project, of which this paper is a part, aims to remedy this by establishing a database of Miocene fungal assemblage distributions, including ecological and climatological tolerances of assemblage members. The FiaWW project was designed to gather globally distributed fossil fungi data and work towards overcoming the difficulties associated with taxonomic identification whenever possible by building collaboration and implementing intensive training between both modern and paleopalynologists, mycologists, and climate scientists. The ultimate goal of the entire FiaWW project is to produce a novel predictive model for fungal assemblage behavior under future warming events by generating a global dataset of fungal assemblages before, during, and after MCO warming that encompasses equatorial, mid-latitude, and high-latitude sites in both hemispheres. As part of this project, fossil fungi extracted from Miocene age sediment have been documented and assigned to modern fungal taxa. The newly acquired Miocene records provide a unique chance to examine fungal assemblages preserved in marine sediments and can be compared with near-coeval assemblages documented on land (e.g., Thailand, Romero et al., 2021). Such comparisons can demonstrate where fungal assemblages are useful as climate proxies and where they are not, a key piece in the development of this new climate change proxy. Recent studies indicate that fungi can be used in Miocene terrestrial settings in the upper midlatitudes (Pound et al., 2022; Worobiec et al., 2022), midlatitudes (O'Keefe et al., 2021; Romero et al., 2021), and tropics (Guimarães et al., 2013; Romero et al., 2021), but it is unknown whether the proxy can translate into high latitudes.

The results presented herein are the first detailed MCO Antarctic fungal record analyzed. Our hypothesis was that fungal diversity and yield would increase to match our plant pollen and spore record during the warm and humid conditions of the MCO.

3 Materials

ANDRILL's southern McMurdo Sound (SMS) project core hole AND-2A, recovered at 77°45.488' S, 165°16.605' E (Fig. 2), represents the only complete proximal record of the

MCO sampled in Antarctica (Acton et al., 2008). The AND-2A core was drilled from a sea ice platform to a total depth of 1138.54 m below the seafloor (m b.s.f.) and recovered over a kilometer of core, which resulted in ~98 % recovery (Acton et al., 2008; Florindo et al., 2008). The cored section is composed of three distinctive stratigraphic intervals from Lower Miocene to Pleistocene. This research is focusing on the Middle Miocene interval (Fig. 2). Biostratigraphy, magnetostratigraphy, and radiometric dating from this core concluded that an approximately 600 m thick Lower Miocene and Middle Miocene section, which includes the MCO, is disconformably overlain at 224.82 m b.s.f. by a thinner Upper Miocene to Pleistocene section (Acton et al., 2008). The lithology changes throughout the deposits reflect variations in sea level, glacial proximity, and climate (Florindo et al., 2008; Harwood et al., 2009). To conduct a comprehensive palynological analysis of the AND-2A core, the science team at McMurdo station, Antarctica, initially collected 230 palynological samples between the depth of 1108 and 28 m b.s.f. at ~5 m intervals. After a preliminary review of the entire sampled interval, 39 additional samples were selected between the depth 578.24 and 310.07 m b.s.f. to provide a high-definition sampling distribution through the entire time interval of the Miocene Climatic Optimum (MCO). For the fungal study discussed herein, a total of 44 samples were analyzed. This includes the set of 39 high-resolution samples (Table 1, set 1) from the original AND-2A study that were re-analyzed for fungal diversity as fungal spores were not the focus of the previous ANDRILL studies. Because the preliminary evaluation of the samples revealed low recovery in fungi, five additional samples (Table 1, set 2) were requested from the Oregon State University Marine and Geology Repository (OSU-MGR) and processed with an acid-free, more gentle technique (see Sect. 4 for details).

4 Methods

4.1 Palynological techniques

All 39 original samples were processed at Global Geolab Limited (Alberta, Canada) following a palynological technique using HCl-HF demineralization, oxidation, neutralization, and sieving (5 µm mesh) protocols outlined in Warny et al. (2009). For each sample preparation, the available amount of dried sediment was weighed out (weights range from 2.4 to 27.3 g), and the palynomorph samples were spiked with a known quantity of *Lycopodium* spores to allow for the quantitative assessment of organic-walled microfossil abundance. As mentioned above, considering that the preliminary evaluation of the samples revealed low recovery in fungi, five additional samples (Table 1, set 2) were processed in the OPaL Lab at Morehead State University with a gentler processing method following deflocculation–sieving and swirling–short centrifugation–heavy-density separation protocols (O'Keefe and Eble, 2012; Pound et al., 2021). Mineral-acid-based

Table 1. Detailed information of total samples analyzed from AND-2A core with corresponding depth, sample processing method, and age correlation from Levy et al. (2016). Samples were processed by two separate methods (set 1: acid-based technique; set 2: gentler technique) to evaluate whether or not the acid-based techniques destroyed some of the more fragile fungal palynomorphs.

Core ID	Slide Set 1	Slide Set 2	Depth (m)
Processing method	Warny et al., 2009	Pound et al., 2021	
126	R-2392-1/COR		310.07
126	R-2150-22/COR		310.51
126		JOMSUGL 1864	310.53
127	R-2392-2/COR		310.97
127	R-2392-3/COR		311.48
127	R-2392-4/COR		312.13
127	R-2392-5/COR		312.85
135	R-2150-24/COR		350.48
140	R-2150-26/COR		372.17
146	R-2150-28/COR		403.48
146	R-2537-22/COR		404.74
146	R-2446-1/COR		406.01
147	R-2446-2/COR		410.2
148	R-2446-3/COR		414.39
148	R-2446-4/COR		418.59
149	R-2446-5/COR		422.79
150	R-2446-6/COR		426.99
151	R-2537-23/COR		430.59
152		JOMSUGL 1865	431.45
152	R-2150-30/COR		431.91
152	R-2537-24/COR		432.18
152		JOMSUGL 1866	432.2
152	R-2537-25/COR		433.02
152		JOMSUGL 1867	433.12
152		JOMSUGL 1868	434
152	R-2392-6/COR		434.02
152	R-2537-26/COR		435.54
153	R-2446-7/COR		439.01
153	R-2537-29/COR		441.01
155	R-2537-27/COR		442.99
155	R-2446-8/COR		444.99
155	R-2537-28/COR		447.07
156	R-2392-7/COR		449.47
156	R-2392-8/COR		450.01
156	R-2392-9/COR		451.01
157	R-2446-9/COR		454.99
157	R-2150-32/COR		458.12
158	R-2392-10/COR		460.12
158	R-2392-11/COR		460.59
163	R-2150-34/COR		485.29
169	R-2150-36/COR		516.49
174	R-2150-38/COR		547.32
177	R-2392-12/COR		558.03
180	R-2150-40/COR		578.24

328.52 m, >15.974 Ma based on normal polarity of C5Cn.1n

358.11 m, 15.91 ± 0.29 Ma based on $^{40}\text{Ar}/^{39}\text{Ar}$ date (lava clast)

366.8 m, 16.05 ± 0.35 Ma based on $^{87}\text{Sr}/^{86}\text{Sr}$ date (*Adamusium* shell)

412.71 m, 16.268 Ma based on MPR of C5Cn.1n/C5Cn.1r

429.56 m, 16.303 Ma based on MPR of C5Cn.1r/C5Cn.2n

434.35 m, 16.23–17.03 Ma based on FAD of *Denticulopsis maccollumii*

434.35 m, 16.77–17.94 Ma based on FAD of *Nitschia* sp. 17

439.23 m, 16.472 Ma based on MPR of C5Cn.2n/C5Cn.2r

440.83 m, 16.54 ± 0.43 Ma based on $^{40}\text{Ar}/^{39}\text{Ar}$ date (lava clast)

448.59 m, 16.543 Ma based on MPR of C5Cn.2r/C5Cn.3n

482.69 m, 16.721 Ma based on MPR of C5Cn.3n/C5Cr

564.92 m, 17.1 ± 0.31 Ma based on $^{40}\text{Ar}/^{39}\text{Ar}$ date (lava clast)

581.34 m, 17.235 Ma based on MPR of C5Cr/C5Dn

methods, usually involving HF, have sometimes resulted in damage to non-pollen palynomorphs, including fungi (van Asperen et al., 2016; Pound et al., 2021). Acid-free methods of palynological processing have demonstrated better preservation of fungal palynomorph structure and features (Clarke, 1994; van Asperen et al., 2016) and generally higher recovery (O’Keefe and Eble, 2012), so making sure the initial processing did not influence recovery was essential.

Fungal spores present in the samples were analyzed using an Olympus BX41 microscope under 60× and 100× oil immersion lenses. A database of fungal spores recovered

was prepared and documented photographically with a digital Q-Capture camera system mounted on the microscope and then managed with Q-Capture (3.1.1) software. Photomicrographs taken were identified and plotted on a stage-based x – y coordinate grid for geolocation. Z -stacked photomicrographs at 100× were constructed using Helicon Focus® to resolve intricate features. Nearest-living-relative (NLR) techniques rely primarily on presence or absence of taxa when applied to dispersed remains (Utescher et al., 2014). The number of fungal palynomorphs produced compared with the number preserved is very poorly understood (Taylor et

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al., 2014; Perrotti et al., 2022). For these reasons, numbers of individual fungal palynomorphs were not counted for every slide in this study, rather, fungal presence–absence was documented. Quantitative counts were made for a few samples to gauge a range in relative fungal abundances. However, due to scarcity of fungal remains, standard palynology counts of 300 grains were unobtainable. Instead, fungal spores and *Lycopodium* spores were counted on every line and on each slide using a transect method to ensure maximum identification of taxa and recovery. Fungal concentration was calculated using the equation of Benninghoff (1962),

$$C = (P_c \times L_t \times T) / (L_c \times W), \quad (1)$$

where C is concentration (specimens per gram of dried weight of sediment sampled, or gdw^{-1}), P_c is the number of fungal spores counted, L_t is the number of *Lycopodium* spores per tablet, T is the total number of *Lycopodium* tablets added per sample, L_c is the number of *Lycopodium* spores counted, and W is the weight of dried sediment (with dried weight (dw) in grams).

An Excel[®] table of dominant palynomorphs recovered was prepared, and key taxa were documented photographically. Taxa were separated by morphology using the following criteria: shape, septation, ornamentation, color, color variation, number, type of apertures, and/or attachment scars (Hughes, 1953; Pirozynski and Weresub, 1979; Elsik et al., 1983; Jansonius and Kalgutkar, 2000; Seifert and Gams, 2011; Guarro, 2012; Gulis et al., 2020; Nuñez Otaño et al., 2021). Taxonomic evaluation was completed utilizing established literature, collections curated at the Louisiana State University Center for Excellence in Palynology (CENEX), digital collections compiled by the FiaWW research group, and a variety of databases to identify associations to family or genus.

4.2 Climate reconstruction techniques

4.2.1 Köppen–Geiger climate classes

The lack of modern-day fungal distribution records produces limitations when reconstructing the paleoenvironment. To resolve this shortcoming, fungal remains have been used in recent studies to reconstruct the climate qualitatively by assigning Köppen–Geiger climate classes to known taxa (Beck et al., 2018). The Köppen–Geiger system utilizes threshold values of modern climatology and seasonality of temperature and precipitation to classify terrestrial areas into five main classes (tropical, arid, temperate, continental, and tundra), and 30 sub-types (Beck et al., 2018). Fungal palynomorphs can thus theoretically be assigned Köppen–Geiger climate classes following the methodology of Pound et al. (2022).

4.2.2 CREST

To reconstruct the regional paleoclimate of Antarctica and better capture uncertainties in climate reconstructions dur-

ing the MCO, pollen data from the Antarctic geologic drilling (ANDRILL) program's AND-2A core in the Ross Sea (Warny et al., 2009), and CREST (Climate Reconstruction SoftWare), a Bayesian (probability-based) paleoclimate reconstruction technique (Chevalier, 2022a) was used. Five bioclimatic variables were assessed in detail: mean annual temperature (MAT), mean temperature of warmest quarter (MTWQ), mean temperature of coldest quarter (MTCQ), mean annual precipitation (MAP), and precipitation seasonality ($\text{CoV} \times 100$). These results provide a new detailed plant-based representation of paleoclimate for the Ross Sea region during the MCO and enable a Köppen–Geiger class to be determined from the pollen data following Beck et al. (2018) for direct comparison with the fungal-based reconstruction.

Because plants are known as accurate climate proxies (Utescher et al., 2014; Chevalier et al., 2020; Spicer et al., 2021), the CREST reconstruction also provides a means of examining the validity of fungal assemblage proxies, as well as a serving for meaningful support for discussion of possible reasons why fungal assemblage as proxy do not work in every setting. Furthermore, CREST provide suitable quantification of error in Middle Miocene and MCO terrestrial climate reconstructions (Romero et al., 2021;) and produces reconstructions consistent with those of the co-existence approach (Gibson et al., 2022). Taxa lists and nearest living relatives were extracted from the literature (Warny et al., 2009; Feakins et al., 2012) and used as the input for CREST. CREST reconstructions were carried out in R using the “crestr” package (RStudio Team, 2022; Chevalier, 2022a). Original documentation of the “crestr” R code is available with the package code (<https://github.com/mchevalier2/crestr>, last access: 28 October 2022).

CREST generates a mean and optimized value and 50 % and 95 % (2σ) uncertainties. Here we focus on mean and the 95 % (2σ) confidence interval. All proxies have assumptions and sources of inherent uncertainty. For paleobotany and palynology these are well explored and documented (e.g., Utescher et al., 2014; Chevalier et al., 2020; Spicer et al., 2021). Specifically, for the MCO of Antarctica, it is likely that the vegetation has no exact extant analogue and the propagule-producing species may be extinct. For these reasons, we have chosen to include the 95 % confidence interval in the presentation of our results to aid with comparisons with other paleobotanical and independent proxy paleoclimate reconstructions of the MCO interval in Antarctica. The reconstructed paleoclimate values, including optima, can be found in Table 2. CREST reconstructed paleoclimate estimates are compared to the modern climatology for McMurdo Station (77.8419° S, 166.6863° E) extracted from WorldClim2.1 (Fick and Hijmans, 2017).

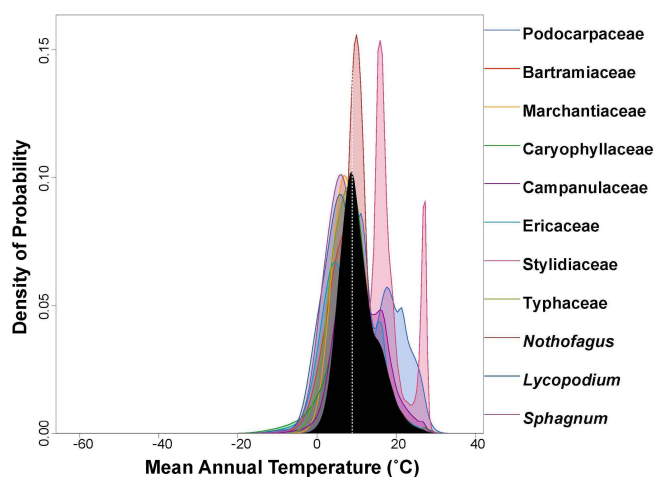


Figure 3. Probability density function (PDF) graphical diagnosis of mean annual temperature (MAT) showing the combination of the PDFs of all the plant taxa recorded in the Andriil2-2A sample. The solid black curve represents the posterior MAT reconstruction, and the dotted white line represents the CREST optima.

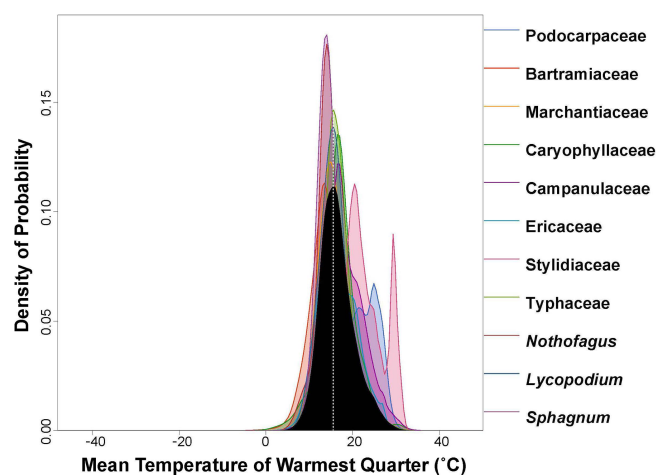


Figure 4. PDF graphical diagnosis of mean temperature of warmest quarter (MTWQ) showing the combination of the PDFs of all the plant taxa recorded in the Andriil2-2A sample. The solid black curve represents the posterior MTWQ reconstruction, and the dotted white line represents the CREST optima.

5 Results

5.1 CREST

CREST requires a minimum of five taxa to work effectively (Chevalier et al., 2014; Chevalier, 2022a). The reconstruction presented herein is based on 13 taxa with a widespread distribution. The palynomorph assemblage used in the paleoclimate reconstruction is thus diverse enough for CREST to use effectively. When compared to the modern-day climate of Antarctica, CREST reconstructs a drastically warmer (by $\sim 28^\circ\text{C}$), drastically wetter (115 % increase in precipitation), and less seasonal eastern Antarctica during the Miocene Climatic Optimum (Table 2). Details of the reconstruction are presented in Figs. 3 to 7. CREST reconstructs a mean annual temperature (MAT) of 10.3°C (2.0 – 20.2°C) with summer temperatures (MTWQ) of 16.4°C (9.3 – 25.3°C) and winter temperatures (MTCQ) of 5.5°C (-5.2 – 18.6°C). Mean annual precipitation (MAP) is reconstructed at 1145 mm yr^{-1} (238 – 2611 mm yr^{-1}) with a seasonality (PS) of 30.0 ($\text{CoV} \times 100$) (6.8 – 68.45). These reconstructions point to a temperate ($^\circ\text{C}$) Köppen–Geiger climate class with a warm summer (b). Although summer and winter precipitation were not reconstructed, the modest nature of PS would imply a Cfb Köppen–Geiger climate class (Table 2).

5.2 The fungal palynological record

The newly acquired fossil fungal record from the AND-2A core shows that fossil fungi are low in both diversity and abundance. Although one processing technique (Pound et al., 2021) is known to be gentler and more optimal for the recovery of fragile palynomorphs, there are no differences ob-

served between the two processed sets, consistent with findings from the Miocene of the UK (Pound et al., 2022). A total of 28 samples are barren, with 16 of those samples appearing consecutively (between the depth of 578.4 and 441.01 m b.s.f.). Only members of the Apiosporaceae, a family that includes a series of genera with simple round brown amero spores ranging in size from 5 to $9\text{ }\mu\text{m}$, were present within those 16 samples (Plate 1). Due to their simple morphology, the fungal spores recovered could only be identified at the family level. The first recovery of these fungal spores occurred at 439.01 m b.s.f., but the presence of fungi is sporadic between 439.01 and 310.07 m b.s.f., except in a consistent interval from 434.02 to 431.91 m b.s.f. The concentration of fungal spores tabulated from those samples where recovery was greatest is very low, ranging between 72 and 199 fungal spores per gram of dried sediment, providing maximum values for fungal abundances during the MCO in southern McMurdo Sound.

Ecological information (Pölme et al., 2020; Zanne et al., 2020) and climatological tolerances (Větrovský et al., 2020) for the genera accepted as part of the Apiosporaceae fungal family are summarized in Table 3.

Despite the taxonomic limitations, all lifestyles, substrates, and habitats associated with the Apiosporaceae genera (Table 3) point to a cosmopolitan group present in all environments outside of dry desert conditions, and extant genera within this fungal family are mainly saprotrophic on wood and plant litter but also include plant pathogens found in aquatic habitats (Pölme et al., 2020).

Table 2. Summary of paleoclimate values reconstructed for southern McMurdo Sound, Antarctica, by CREST on the basis of palynological results from AND-2A.

Bioclimatic variable	ANDRILL-2A						Modern
	Optima	Mean	Lower 50	Upper 50	Lower 95	Upper 95	McMurdo Station
MAT (°C)	8.7	10.3	6.0	11.5	2.0	20.2	−17.6
MTWQ (°C)	15.6	16.4	13.0	17.7	9.3	25.3	−5.0
MTCQ (°C)	4.0	5.5	0.6	7.4	−5.2	18.6	−25.6
MAP (mm yr ^{−1})	753	1145	544	1076	238	2611	302
PS (CoV × 100)	21.1	30.0	15.0	28.6	6.8	68.5	61.0

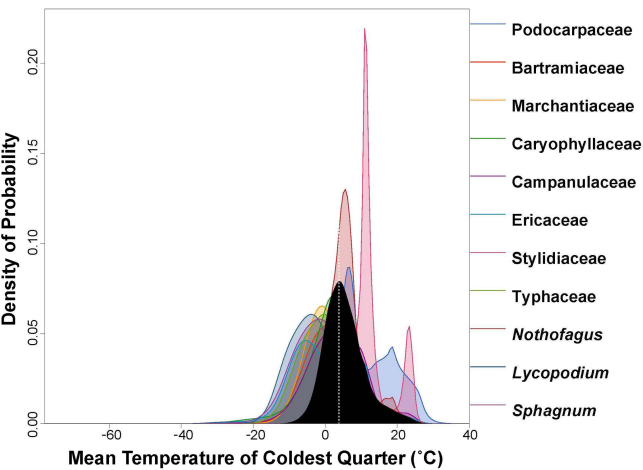


Figure 5. PDF graphical diagnosis of mean temperature of coldest quarter (MTCQ) showing the combination of the PDFs of all the plant taxa recorded in the Andrill2-2A sample. The solid black curve represents the posterior MTCQ reconstruction, and the dotted white line represents the CREST optima.

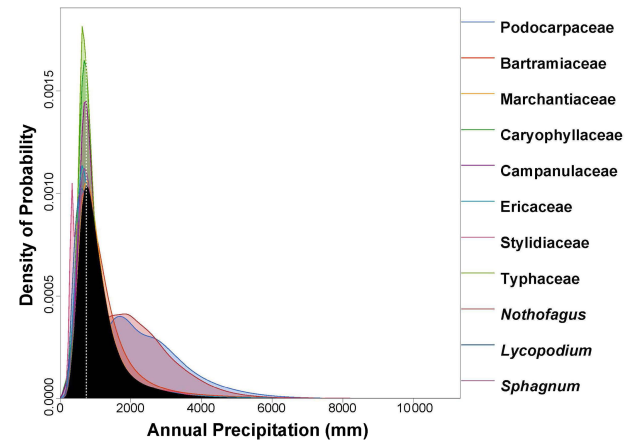


Figure 6. PDF graphical diagnosis of mean annual precipitation (MAP) showing the combination of the PDFs of all the plant taxa recorded in the Andrill2-2A sample. The solid black curve represents the posterior MAP reconstruction, and the dotted white line represents the CREST optima.

5.3 Fungal-spore-based climatological inferences

Despite the limitations noted above, online databases were reviewed for all locations where Apiosporaceae are found today and each location was assigned the associated Köppen–Geiger climate class. This fungal-based reconstruction was made for the 16 samples from SMS that contained fossil fungi. Since each sample contained only Apiosporaceae, their Köppen–Geiger climate class reconstructions were identical. A total of 23 climate classes appear within the reconstruction, including 5 broad classes: A (tropical), B (arid), C (temperate), D (cold), and E (polar), each with their own sub-groups. This means that this specific taxon is found in a broad variety of climates today. Thus, the widely cosmopolitan nature of Apiosporaceae means that for our site the fungi-based climate reconstruction for SMS is of no value. It only shows that the fungi entering the fossil record during the MCO in Antarctica are those tolerant of a wide range of climatic conditions. The same trait was observed for the dinoflagellate assemblages, in which *Operculodinium centrocarpum*, a known cosmopolitan alga, was the main species able to recolonize the Antarctic coastal waters during the MCO.

6 Discussion

6.1 Palynology

This study focused on describing fossil fungal composition from southern McMurdo Sound with a trait-based morphological approach. During the time interval analyzed, an important recolonization of plants was observed at the SMS site (Warny et al., 2009). The most abundant plant palynomorphs recovered indicate a proliferation of woody plants, specifically a variety of *Nothofagus* species (Southern beech trees) and Podocarpaceae (podocarp conifer). Also observed were other components of the vegetation, including bryophytes (mainly *Coptospora* spp., moss, Bartramiaceae), Marchantiaceae (liverworts), a *Lycopodium* (clubmoss) similar to an extant Patagonian species, and angiosperms characteristic of modern subantarctic and austral-alpine environments such as Caryophyllaceae (*Colobanthus* type, pearlwort), Styliidiaceae (trigger plants), Droseraceae (sundews), Campanu-

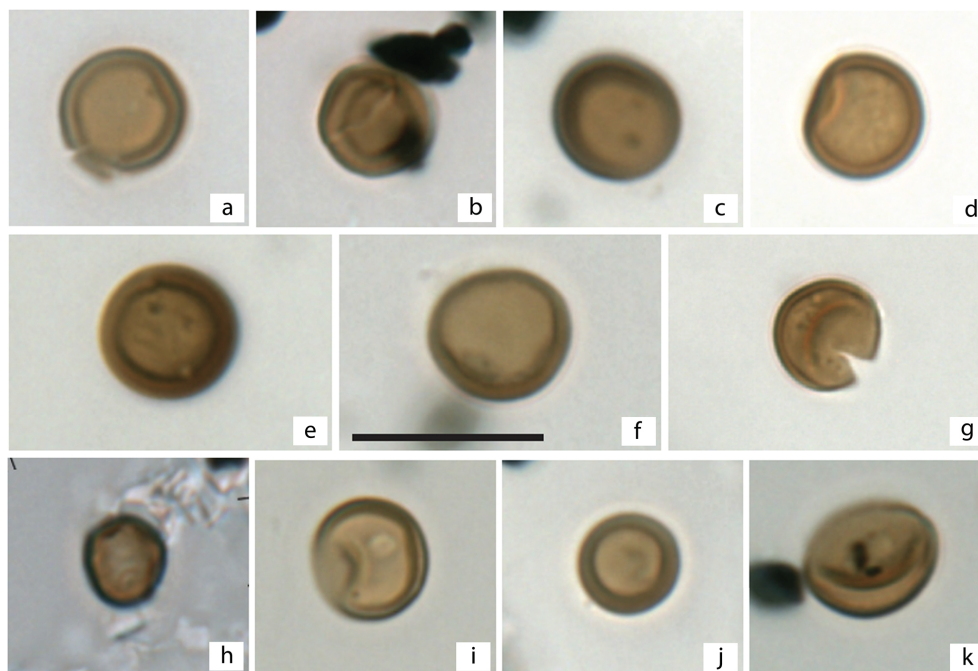


Plate 1. Fungal spores recovered from the AND-2A Core, southern McMurdo Sound, Antarctica. (a)–(k) Apiosporaceae. The scale bar is 10 μ m.

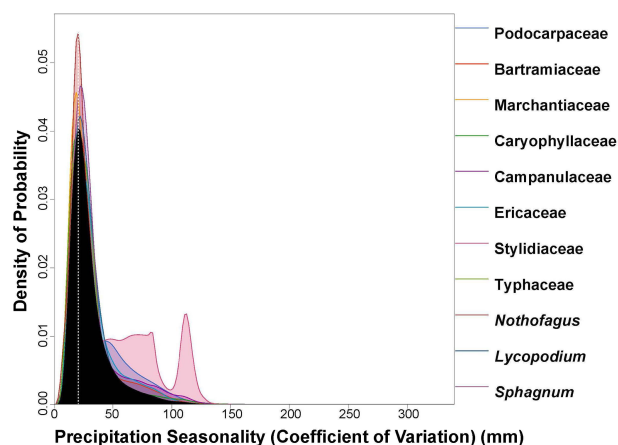


Figure 7. PDF graphical diagnosis of precipitation seasonality ($\text{CoV} \times 100$) showing the combination of the PDFs of all the plant taxa recorded in the Andriil2-2A sample. The solid black curve represents the posterior Precipitation Seasonality reconstruction, and the dotted white line represents the CREST optima.

laceae (bell flowers), Ericales (heaths), Poaceae (grasses), and Typhaceae and/or Sparganiaceae (bullrushes, burr reeds).

This new study of the fungal fraction of the SMS site indicates that, while vegetation during the MCO was somewhat abundant and diverse, fungal recovery from Antarctica during the Middle Miocene was sparse and only represented by spores from a single family, Apiosporaceae, of which a member (*Spegazzinia*) is extant along the Antarctic

coast and in southernmost Patagonia today (Musotto et al., 2017). Apiosporaceae are endophytes, parasites, and saprophytes and have a cosmopolitan distribution, except in dry desert climates. This trait and the associated adaptability of the group is probably the reason why they were the one group able to thrive during the brief episode of improved climatic conditions in Antarctica. These fungi were likely associated with the various forms of plant remains (leaves, wood) that thrived during the MCO interval.

Despite the poor recovery, this study provides the first record of fossil fungal spores from Antarctic MCO sediments (Fig. 8). This information is useful for paleoecological inferences concerning relative abundance of decaying plant remains accumulated during favorable climatic conditions of MCO.

6.2 CREST

The CREST analysis provides a maximum mean annual temperature of 10.3 $^{\circ}\text{C}$ and a mean annual precipitation of 1147 mm yr^{-1} for the warmest intervals of the MCO (Table 2 and Fig. 9). The reconstructed MCO climate of ANDRILL-2A is considerably warmer and wetter than the modern climate of the nearby McMurdo Station. The modern polar-desert conditions at McMurdo have an average annual temperature of -17.6°C with summer averages of -5.0°C and winter averages of -25.6°C . Compared to the modern values, the reconstructed MCO estimates represent a 27.9 $^{\circ}\text{C}$ increase in annual average temperature, a 21.4 $^{\circ}\text{C}$ increase

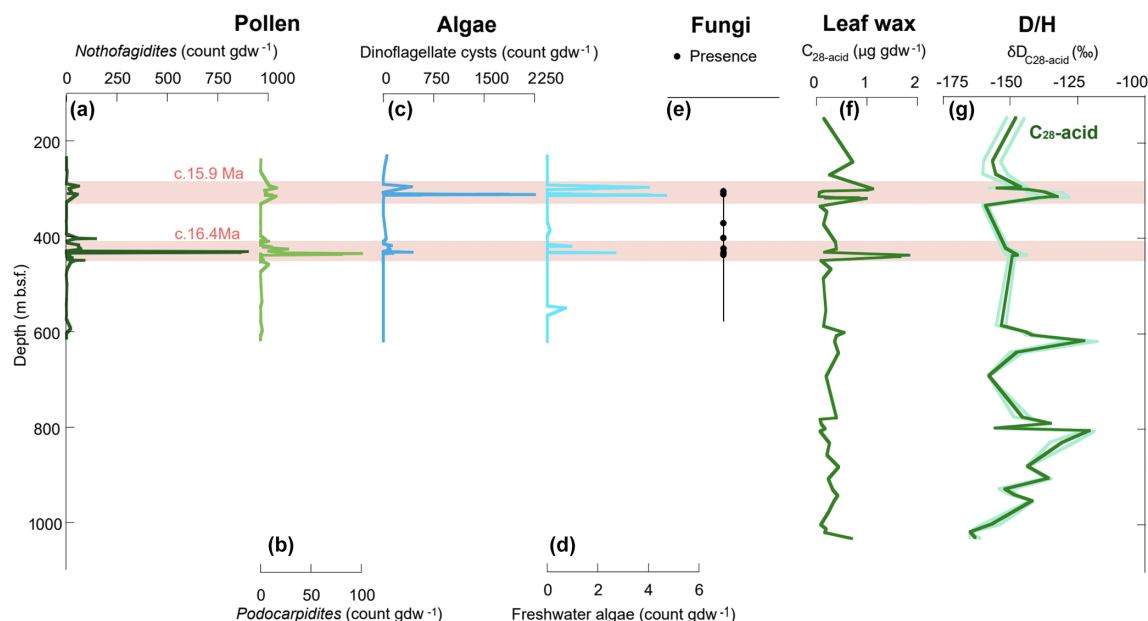


Figure 8. Geochemical and palynological results from AND-2A core. **(a)** *Nothofagidites* pollen concentration (cc). **(b)** *Podocarpidites* pollen cc. **(c)** Dinoflagellate cyst cc. **(d)** Freshwater algae cc. **(e)** Presence of Apiosporaceae fungal spores from this study (note that each dot represents several non-barren samples). **(f)** Leaf wax abundance. **(g)** δD , mean (dark green), 1 standard deviation (light green). Pink shading highlights two peaks identified in abundance data (modified after Feakins et al., 2012).

in summer temperatures, and a 10.5°C increase in winter temperatures. Modern average annual precipitation at McMurdo is low at 302 mm yr^{-1} and precipitation seasonality is 61.0 ($\text{CoV} \times 100$), meaning the MCO reconstruction is 279 % wetter and approximately half as seasonal.

The CREST reconstruction aligns with the interpretation of a warmer, wetter, less seasonal world during the MCO (Steinhorsdottir et al., 2021) and is supported by independent proxy evidence from the Ross Sea and penecontemporaneous records from across Antarctica. Northern high-latitude areas were potentially 14°C warmer than preindustrial times during the MCO (Pound et al., 2012) and it has been suggested that southern high latitudes were similarly warmed (Pagani et al., 2011; Steinhorsdottir et al., 2021). The reconstruction presented here suggests the southern high latitudes experienced enhanced warming with average summer temperatures reconstructed over 20°C warmer and winter temperatures 10°C warmer than preindustrial times. Considered in the Köppen–Geiger climate classification scheme, the MCO is reconstructed as a Cfb climate class (temperate, no dry season, warm summer), as opposed to the EF climate class (polar, frost) of present-day SMS (Beck et al., 2018). The nearest present-day location to SMS with this Köppen–Geiger climate classification is the Snares Islands of New Zealand at 48°S (Beck et al., 2018).

The relative warming reconstructed by CREST is supported by various proxy records from the ANDRILL-2A core and beyond. The encroachment of plant taxa associated with lower latitudes today, specifically *Nothofagites*, has been re-

peatedly linked to warming during the Neogene (e.g., Lewis et al., 2008; Warny et al., 2009; Anderson et al., 2011; Sanguigni et al., 2018). The taxonomic composition of terrestrial palynomorphs from the ANDRILL-2A record, including the large increase in the relative abundance of *Podocarpidites* (podocarp conifer) and *Nothofagidites* (southern beech) suggests austral summer temperatures reached at least 10°C during the MCO (Warny et al., 2009), similar to average annual temperature estimated from pollen assemblages from ANDRILL-2A of $0\text{--}10^{\circ}\text{C}$ (Feakins et al., 2012). Dinoflagellate species from ANDRILL-2A show a replacement of sea ice indicator taxa by temperate taxa, also indicating similar temperatures within the range of 0 to 11.5°C in the Ross Sea (Warny et al., 2009). These temperatures are close to the CREST reconstructed MAT of 10.3°C . Marine sediments from ANDRILL-2A have yielded plant wax δD that suggest MCO summer temperatures of 7°C on land (Feakins et al., 2012). These summer temperatures are lower than MTWQ (summer) reconstructed by CREST (16.4°C ($9.3\text{--}25.3^{\circ}\text{C}$)) and more comparable to MAT (10.3°C ($2.0\text{--}20.2^{\circ}\text{C}$)).

CREST temperature reconstructions align well with sea surface temperature (SST) reconstructions from the Ross Sea and surrounding regions. SSTs from peak warming during the MCO (ca. 15.7 Ma) suggest a warming of $6\text{--}10^{\circ}\text{C}$ in the Ross Sea (Levy et al., 2016). This is well within the 27.9°C warming reconstructed by CREST for MAT relative to preindustrial times but more comparable with the 10.5°C increase for MTCQ (winter). In addition, TEX86 from ANDRILL-2A from the Ross Sea (Levy et al., 2016)

recalibrated with BAYSPAR yields SST estimates of ~ 11 °C (6–26 °C) spanning from 20 to 11 Ma, which are within the range of the 95 % confidence interval for CREST MAT and MTWQ (summer) reconstructions.

Slightly further afield in the Dry Valleys of the Transantarctic Mountains, cypridoidean ostracods and lathridiid beetle fossils yield summer temperatures of 5 °C immediately following the Mid-Miocene Climate Transition (MMCT) (14 Ma) (Lewis et al., 2008) more comparable with CREST reconstructed MTCQ (winter) and indicative of post MCO cooling. TEX86 from DSDP Site 269 reveals warm ocean temperatures of up to 14 °C persisted off Wilkes Land, East Antarctica (Evangelinos et al., 2020), and at IODP Site U1356A (~ 60° S paleolatitude), the Methylation of Branched Tetraether (MBT)/Cyclization of Branched Tetraether (CBT) method has estimated Miocene Wilkes Land MAT as 12 °C for 16.3 to 13.8 Ma (Sangiorgi et al., 2018). These are comparable with the CREST MTWQ (summer) reconstruction.

Penecontemporary evidence from elsewhere in Antarctica provides similar temperature reconstructions. Branched glycerol dialkyl glycerol tetraethers from soil bacteria suggest air temperatures of 12 °C ± 1 °C for months above freezing for the Miocene with TEX86 Miocene SSTs ranging from 14.3 to 3.9 °C (Tibbett et al., 2022), which are similar to CREST reconstructions of mean MTWQ (summer) and MTCQ (winter), respectively. TEX86 BAYSPAR SSTs from IODP Site U1356 (60° S) in the Weddell Sea indicate SSTs of 12 °C ± 3 °C (13.8–10.8 Ma) (Hartman et al., 2018), which is within the 95 % confidence intervals of CREST MAT, MTWQ (summer), and MTCQ (winter). SSTs have also been reconstructed at IODP Site 1171 (48°30' S, 149°6' E) based on clumped isotopes measured on planktonic foraminiferal calcite yielding 12.7 °C ± 1.1 °C (Leutert et al., 2020), which are within the 95 % uncertainties for CREST MAT, MTWQ, and MTCQ. The warmest temperatures are reconstructed from offshore ODP Site 1171 (55° S) on the South Tasman Rise where TEX₈₆ BAYSPAR indicates SSTs of 22.6 °C ± 2.5 °C (15.3–12.0 Ma) (Leutert et al., 2020), which is within the 95 % confidence interval for CREST MTWQ (summer).

7 Conclusions

The goal of this study is to provide a new climate reconstruction using plant-based fossils and to evaluate fungal composition during the MCO in Antarctica. New plant-based paleoclimate reconstructions performed using CREST indicate a warmer and wetter than present paleoclimate that agrees with previous studies completed using various geochemical proxies, with mean annual temperature and precipitation reconstructed at 10.3 °C and 1145 mm yr⁻¹, respectively. The plant-based reconstructions define a temperate, no dry season with a warm summer (Cfb) Köppen–Geiger climate classi-

Table 3. Key genera of the Apiosporaceae family according to three databases (Indexfungorum, Mycobank, and FungalTraits) and their modern ecological affinities.

Phylum	Class	Order	Family	Genus	Primary lifestyle	Secondary lifestyle	Plant pathogenic capacity	Decay substrate	Aquatic habitat	Growth form	Fruit body type	Hymenium type
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Appendicospora</i>	saprotroph			wood	non-aquatic	filamentous mycelium	perithecium (hymenium hidden, narrow opening)	closed
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Apiospora</i>	plant pathogen			partly aquatic	filamentous mycelium			
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Arthrinium</i>	plant pathogen	foliar endophyte	leaf/fruit/seed pathogen	leaf/fruit/seed	partly aquatic	filamentous mycelium	perithecium (hymenium hidden, narrow opening)	closed
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Endoclix</i>	wood saprotroph			wood	non-aquatic	filamentous mycelium	perithecium (hymenium hidden, narrow opening)	closed
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Nigrospora</i>	litter saprotroph		leaf/fruit/seed pathogen	leaf/fruit/seed	partly aquatic	filamentous mycelium	perithecium (hymenium hidden, narrow opening)	closed
Ascomycota	Sordariomycetes	Xylariales	Apiosporaceae	<i>Scyphospora</i>	wood saprotroph			wood	non-aquatic	filamentous mycelium	perithecium (hymenium hidden, narrow opening)	closed

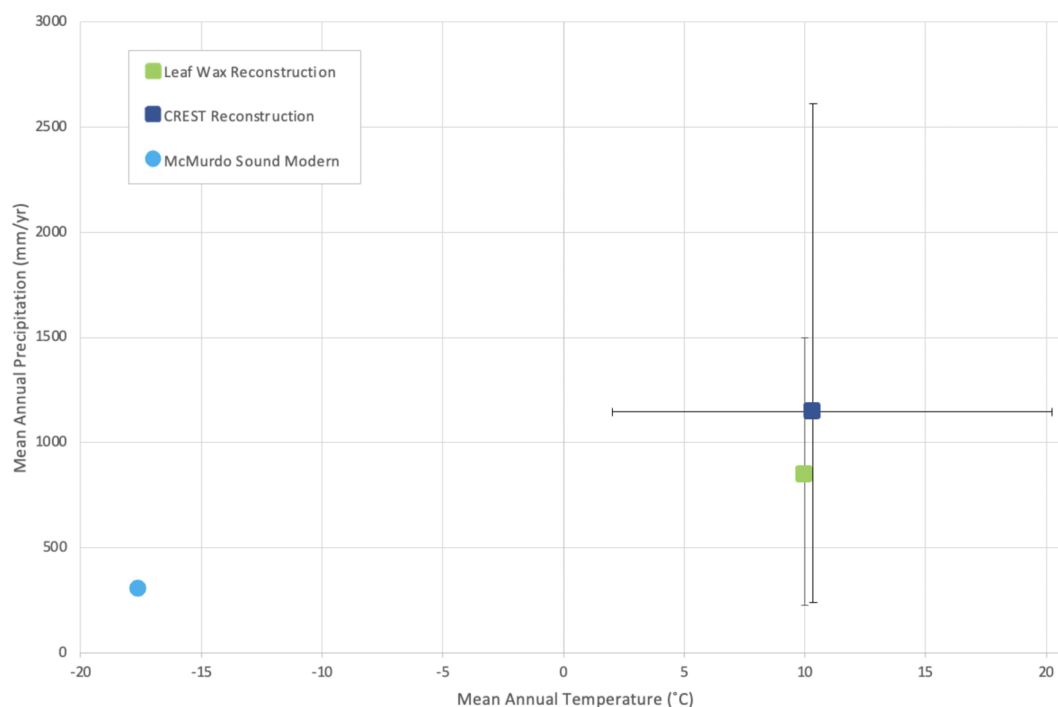


Figure 9. Mean annual temperature vs. mean annual precipitation of southern McMurdo Sound, Antarctica. Error bars represent the 95 % (2σ) confidence interval. Squares indicate Miocene climate reconstructed by CREST (this study, dark blue) and leaf wax (Feakins et al., 2012, green), and the circle indicates modern-day climate in southern McMurdo Sound, Antarctica.

fication during the MCO for southern McMurdo Sound. In the present study, the MCO in the Ross Sea region is reconstructed 279 % wetter than modern conditions, representing a relative 843 mm yr^{-1} increase in precipitation. This increase agrees with precipitation reconstructed from leaf wax hydrogen isotope data from the ANDRILL-2A core (Feakins et al., 2012).

The 44 samples analyzed from the ANDRILL-2A core in southern McMurdo Sound ranged in age from approximately 17 to 15 Ma, but fungal spores were only present from ~ 16.4 to 15.6 Ma. Results described herein show a poor recovery of fungal spores with a maximum concentration per gram of dried sediment ranging up to ~ 200 , as opposed to a concentration of up to ~ 900 for pollen, and up to ~ 2250 for dinoflagellates. Only 36 % of samples analyzed yielded fungal spores or fungal remains, all from a single family, the Apiosporaceae. The presence of this single type, a saprotrophic cosmopolitan family that inhabits substrates rich in both woody and herbaceous plants, some of which are associated with freshwater habitats, supports the assumption that these fungi were able to recolonize some areas of Antarctica during this brief interval of warming and increased moisture availability. Unfortunately, the limited fossil fungi record recovered prevents the use of fungi for fungal-based paleoclimatic reconstruction.

Code availability. The code used within this work is publicly available here <https://cran.r-project.org/web/packages/crestr/index.html> (last access: 28 October 2022) and here <https://github.com/mchevalier2/crestr> (last access: 28 October 2022). An older format is also available at <https://doi.org/10.5281/zenodo.6458405> (Chevalier, 2022b).

Data availability. Data sets used in this study are available upon request.

Author contributions. MP conducted the fungal analyses, MEG conducted all CREST analyses, SW conducted the pollen and spore analyses and advised MP during her MS thesis. NBNO and ICR provided guidance and training on fungal taxonomy. MJP and JMKO are the lead PIs on the FiaWW project and provided guidance to MP and MEG. All authors contributed in various ways to the writing of the paper.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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