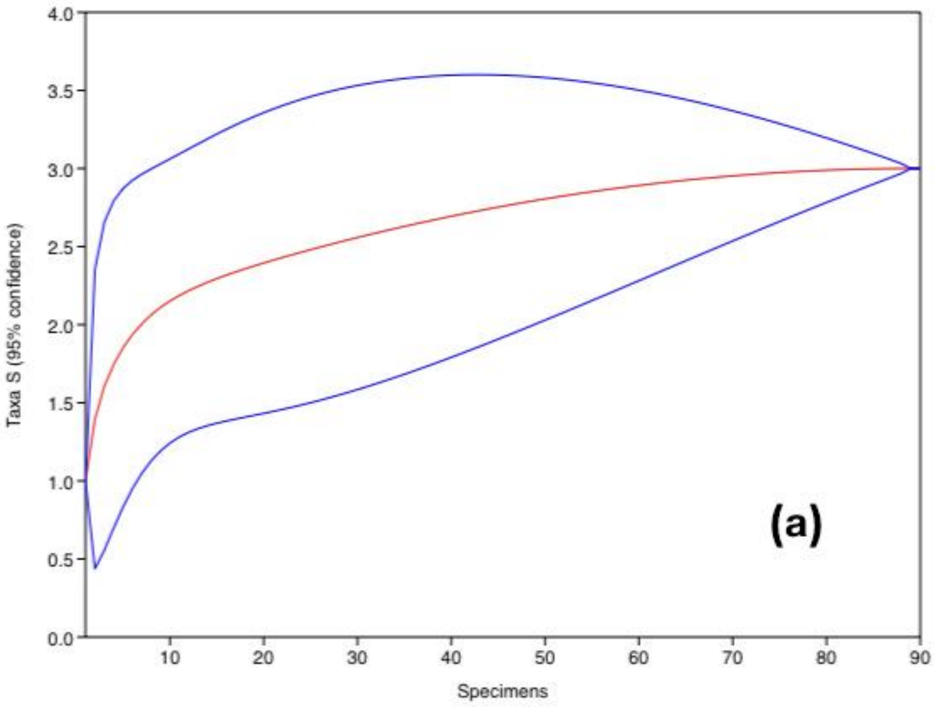
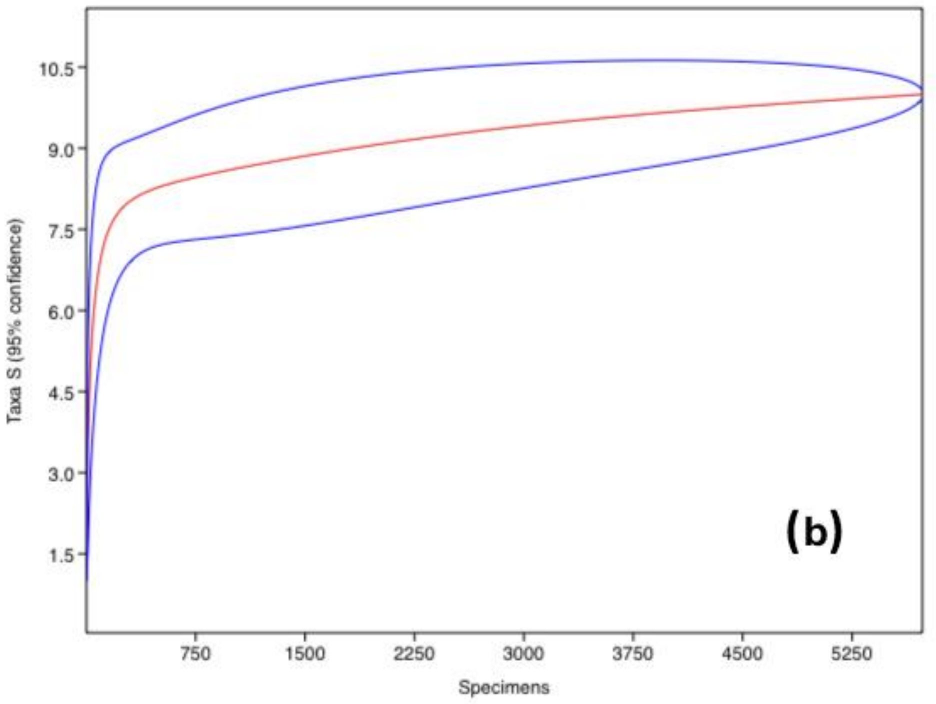
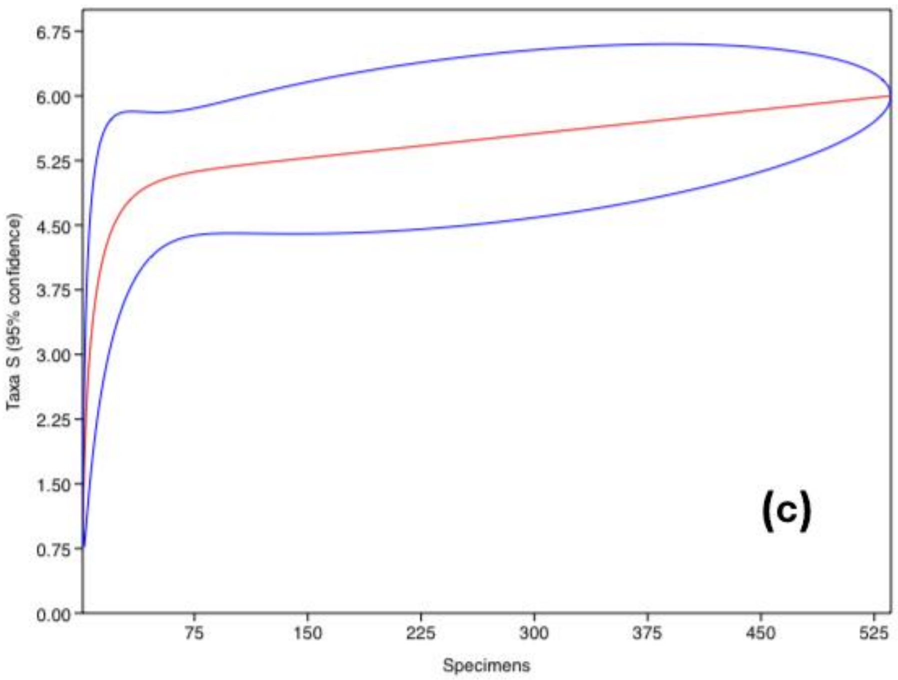
Supplementary material for: Câmara P.E.A.S., Šantl-Temkiv T., Pinto O.H.B., Convey P., Dall'Osto M., Bones F.L.V., Carvalho-Silva M., Knop Henriques D. & Rosa L.R. 2025. DNA metabarcoding of non-fungal eukaryotic diversity in air and snow of Livingston Island, South Shetland Islands, Antarctica. *Polar Research 44*. Correspondence: Paulo E.A.S. Câmara, Departamento de Botânica, Universidade de Brasília, Brasília, Campus Universitário Darcy Ribeiro, Asa Norte, Brazil, 70910-900. E-mail: paducamara@gmail.com.







'**Supplementary Fig. S1.** Rarefaction curves based on taxa profile (97% similarity cutoff) from (a) air, (b) Snow1 and (c) Snow2 samples. Blue lines represent 95% confidence limits inferred using bootstrap values calculated from 1000 iterations using PAST, version 1.90.

Methodological details

The methods used here were the same as those used and cited by Rosa et al. (2020).

DNA extraction

DNeasy PowerSoil Kit (Qiagen, USA) following the manufacturer's instructions. DNA quality was analysed by agarose gel electrophoresis (1% agarose in 1 X Trisborate-EDTA) and then quantified using the Quanti-iT ™ Pico Green dsDNA Assay (Invitrogen). Extracted DNA was used as template for generating PCR amplicons. PCR amplicons were generated using the universal primers ITS3 and ITS4 and were sequenced by high-throughput sequencing at Macrogen Inc. (South Korea) on an Illumina MiSeq sequencer, using the MiSeq Reagent Kit version 3 (600-cycle) following the manufacturer's protocol.

Data analyses and taxa identification

Quality analysis was carried out using BBDuk version 38.87 in BBmap software (Bushnell 2014) with the following parameters: Illumina adapters removing (Illumina artefacts and the PhiX Control v3 Library); ktrim = l; k = 23; mink = 11; hdist = 1; minlen = 50; tpe; tbo; qtrim = rl; trimq = 20; ftm = 5; maq = 20. The remaining sequences were imported to QIIME2 version 2021.4 (https:// qiime2.org/) for bioinformatics analyses (Bolyen et al.

2019). The qiime2-dada2 plugin was used for filtering, dereplication, turn paired-end fastq files into merged, and remove chimeras, using default parameters (Callahan et al. 2016).

Taxonomic assignments of amplicon sequence variants (ASVs) were determined using the qiime2-feature-classifier (Bokulich et al. 2018) classify-sklearn against different databases, using a sequence similarity threshold of 97%. First, ASVs were classified against the PLANiTS2 database (Banchi et al. 2020). After this step, ASVs that remained unclassified were filtered and classify-sklearn classified against the UNITE Eukaryotes ITS database version 8.3 (Abarenkov et al. 2020).

Finally, remaining unclassified ASVs were filtered and aligned against the filtered National Center for Biotechnology Information (NCBI) non-redundant nucleotide sequences (nt) database (February 2020) using BLASTn (Camacho et al. 2009) with default parameters; the nt database was filtered with the following keywords: “ITS1”, “ITS2”, “Internal transcribed spacer”, and “internal transcribed spacer”.

Taxonomic assignments were performed using MEGAN6 (Huson et al. 2016). For simplicity we henceforth refer to the assigned ASVs as “taxa”. Venn diagrams were prepared as described by Bardou et al. (2014).

For comparative purposes, we consider reads as a proxy for abundance (Deiner et al. 2017, Hering et al. 2018; Câmara, Carvalho-Silva et al. 2021; Câmara, Convey et al. 2021; Carvalho-Silva et al. 2021; Rosa et al. 2021; Câmara et al. 2022).

Diversity analyses

Rarefaction calculations were carried out using the rarefaction analysis command in the software PAST 4.03 (Hammer et al. 2001). Venn diagrams were prepared as described by Bardou et al. (2014).

Supplementary Table S1. Raw data and statistics. All databases were consulted in February 2020.

DNA reads	Total	Database consulted		
		PLANiTS2[a]	UNITE[b]	NCBI[c]
Number of raw reads	751 670			
Reads after filtering	439 168			
Reads from fungi (Rosa et al. 2020)	430 748			
Reads from non-fungal organisms	8420			
Unassigned reads	2054			
Known reads from non-fungal organisms	6366			
Reads from air sample	90	69	21	0
Reads from snow samples	6276	5480	238	46

[a] https://github.com/apallavicini/PLANiTS. [b] https://unite.ut.ee/. [c] National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov.

References

Abarenkov K., Zirk A., Piirmann T., Pöhönen R., Ivanov F., Nilsson H.R. & Kõljalg U. 2020. UNITE QIIME Release for Eukaryotes 2. Version 4: 2020. (Data set.) Doi: 10.15156/BIO/786388.

Banchi E., Ametrano C.G., Greco S., Stanković D., Muggia L. & Pallavicini A. 2020. PLANiTS: a curated sequence reference dataset for plant ITS DNA metabarcoding. *Database 2020*, baz155, doi: 10.1093/database/baz155.

Bardou P., Mariette J., Escudié F., Djemiel C. & Klopp C. 2014. Jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics 15*, article no. 293, doi: 10.1186/1471-2105-15-293.

Bokulich N.A., Kaehler B.D., Rideout J.R., Dillon M., Boylern E., Knight R., Huttley G.A. & Caporaso J.G. 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome 6*, 90-107, doi: 10.1186/s40168-018-0470-z.

Bolyen E., Rideout J.R., Dillon M.R., Bokulich N.A., Abnet C.C., Al-Ghalith A., Alexander, H., Alm E.J., Arumugan M., Asnicar F., Bai Y., Bisanz J.E., Bittinger K., Brejnrod A., Brislawn C.J., Brown C.T., Callahan B.J., Chase J., Cope E.K., Silva R., Diener C., Dorrestein P.C., Douglas G.M., Durall D.M., Duvallet C., Edwardson C.F., Ernst M., Estaki M., Fouquier J., Gauglitz J.M., Gibbons S.M., Gibson D.L., Gonzales A., Gorlick K., Guo J., Hillmann B., Holmes S., Holste H., Huttenhower C., Huttley G.A., Janssen S., Jarmusch A.K, Jiang L., Kaehler B.D., Kang K.B., Keefe C.R., Keim P., Kelley S.T., Knights D., Koester I., Kosciolek T., Kreps J., Langille M.G.I., Lee J., Ley R., Liu Y., Loftfield E., Lozupone C., Maher M., Marotz C., Martin B.D., McDonald D., McIver L.J., Melnik A.V., Metcalf J.L., Morgan S.C., Morton J.T., Naimey A.T., Navas-Molina J.A., Nothias L.F., Orchanian S.B., Pearson T., Peoples S.L., Petras D., Preuss M.L., Pruesse E., Rasmussen L.B., Rivers A., Robeson I.I., MS, Rosenthal P., Segata N., Shaffer M., Shiffer A., Sinha R., Song S.J., Spear JR, Swafford A.D., Thompson L.R., Torres P.J., Trinh P., Tripathi A., Turnbaugh P.J., Ul-Hasan S., van der Hoof J.J.J., Vargas F., Vazques-Baeza Y., Vogtmann E., von Hippel M., Walters W., Wan Y., Wang M., Warren J., Weber K.C., Williamson C.H.D., Willis A.D., Xu Z.Z., Zaneveld J.R., Zhang Y., Zhu Q., Knight R. & Caporaso J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology 37*, 852–857, doi: 10.1038/s41587-019-0209-9.

Bushnell B. 2014. BBMap: a fast, accurate, splice-aware aligner. Berkeley, CA: Lawrence Berkeley National Lab. Accessed on the internet at https://sourc eforge.net/proje cts/bbmap

Callahan B.J., McMurdie P.J., Rosen M.J., Han A.W., Johnson A.J.A. & Holmes S.P. 2016. DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods 13*, 581–583, doi: 10.1038/nmeth.3869.

Camacho C., Coulouris G., Avagyan V., Ma N., Papadopoulos J., Bealer K. & Madden T.L. 2009. BLAST+: architecture and applications. *BMC Bioinformatics 10*, article no. 421, doi: 10.1186/1471-2105-10-421.

Câmara P.E.A.S., Carvalho-Silva M., Pinto O.H.B., Amorim E.T., Henriques D.K., Silva, T.H., Pellizzari F., Convey P. & Rosa L.H. 2021. Diversity and ecology of Chlorophyta

(Viridiplantae) assemblages in protected and non-protected sites in Deception Island (Antarctica, South Shetland Islands) assessed using an NGS approach. *Microbial Ecology 81*, 323–334, doi: 10.1007/s00248-020-01584-9.

Câmara P.E.A.S., Convey P., Rangel S.B., Konrath M., Barreto C.C., Pinto O.H.B., Carvalho-Silva M., Henriques D.K., Oliveira H.C. & Rosa L.H. 2021. The largest moss carpet transplant in Antarctica and its bryosphere cryptic biodiversity. *Extremophiles 25*, 369–384. doi: 10.1007/s00792-021-01235-y.

Câmara, P.E.A.S., de Menezes, G.C.A., Oliveira, F.S., Delpuppo C., Schaefer C.E.G.R, Convey P., Pinto O.H.B., Carvalho-Silva M. & Rosa L.H. 2022. Diversity of Viridiplantae DNA present on rock surfaces in the Ellsworth Mountains, continental Antarctica. *Polar Biology 45*, 637–646. doi: 10.1007/s00300-022-03021-8.

Carvalho-Silva M., Rosa L.H., Pinto O.H.B., Da Silva T., Henriques D.K., Convey P. & Câmara, P.E.A.S. 2021. Exploring the plant environmental DNA diversity in soil from two sites on Deception Island (Antarctica, South Shetland Islands) using metabarcoding. *Antarctic Science 33*, 469-478, doi: 10.1017/S0954102021000274.

Deiner K., Bik H.M., Mächler E., Seymour M., Lacoursière-Roussel A., Altermatt F., Creer S., Bista I., Lodge DM, de Vere N., Pfrende, M.E. & Bernatchez L. 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. *Molecular Ecology 26*, 5872-5895, doi: 10.1111/mec.14350.

Hammer Ø., Harper D.A.T. & Ryan P.D. 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica 4*, article no. 4.

Hering D., Borja A., Jones J.I., Pont D., Boets P., Bouchez A., Bruce, K. Drakare S., Hanfling B., Kahlert M., Leese F., Meissner K., Mergen P., Reyjol Y., Segurado P., Vogler A. & Kelly M., 2018. Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. *Water Research 138*, 192-205, doi: 10.1016/j.watres.2018.03.003.

Huson D.H., Beier S., Flade I., Górska A., El-Hadidi, Mitra S., Ruscheweyh H.J. & Tappu R. 2016. MEGAN community edition—interactive exploration and analysis of large-scale microbiome sequencing data. *PLOS Computational Biology 12*, e1004957, doi: 10.1371/journal.pcbi.1004957.

Rosa L.H., Pinto O.H.B., Šantl-Temkiv T., Convey P., Carvalho-Silva M. Rosa, C.A. & Rosa L.H. 2020. DNA metabarcoding of fungal diversity in air and snow of Livingston Island, South Shetland Islands, Antarctica. *Scientific Reports 10*, article no. 21793, doi: 10.1038/s41598-020-78630-6.

Rosa L.H., Pinto O.H.B., Convey P., Carvalho-Silva M., Rosa, C.A. & Câmara P.E.A.S. 2021. DNA metabarcoding to assess the diversity of airborne fungi present over Keller Peninsula, King George Island, Antarctica. *Microbial Ecology 82*, 165–172, doi: 10.1007/s00248-020-01627-1.