Fungal Biology 127 (2023) 997–1003

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

# Soil fungal diversity and assembly along a xeric stress gradient in the central Namib Desert

S. Vikram [a], J.-B. Ramond [a, b], M. Ortiz [a], G. Maggs-Kölling [c], K. Pelser [a], D.A. Cowan [a, *]

[a] Centre for Microbial Ecology and Genomics, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, Pretoria, South Africa
[b] Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, Chile
[c] Gobabeb-Namib Research Institute, Walvis Bay, Namibia

ARTICLE INFO

*Article history:*
Received 27 October 2022
Received in revised form
2 March 2023
Accepted 6 March 2023
Available online 7 March 2023

Handling Editor: Yuyi Yang

*Key Words:*
Namib desert
Hyperarid
Edaphic
Fungal diversity and assembly
Xeric gradient

ABSTRACT

The Namib Desert of south-western Africa is one of the oldest deserts in the world and possesses unique geographical, biological and climatic features. While research through the last decade has generated a comprehensive survey of the prokaryotic communities in Namib Desert soils, little is yet known about the diversity and function of edaphic fungal communities, and even less of their responses to aridity. In this study, we have characterized soil fungal community diversity across the longitudinal xeric gradient across the Namib desert (for convenience, divided into the western fog zone, the central low-rainfall zone and the eastern high-rainfall zone), using internal transcribed sequence (ITS) metabarcoding. Ascomycota, Basidiomycota and Chytridiomycota consistently dominated the Namib Desert edaphic fungal communities and a core mycobiome composed of only 15 taxa, dominated by members of the class Dothideomycetes (Ascomycota), was identified. However, fungal community structures were significantly different in the fog, low-rainfall and high-rainfall zones. Furthermore, Namib Desert gravel plain fungal community assembly was driven by both deterministic and stochastic processes; the latter dominating in the all three xeric zones. We also present data that suggest that the inland limit of fog penetration represents an ecological barrier to fungal dispersal across the Namib Desert.


## 1. Introduction

The microbial ecology of the coastal Namib Desert of western Namibia (south-western Africa) has, until the last decade, received relatively little research attention (Cowan et al., 2020). Nevertheless, the age of this desert (~43 million y old; Seely and Pallett, 2012), the unusual west-to-east coastal fog to inland rain precipitation dynamics (Eckardt et al., 2013), the clear demarcation into two geomorphological zones (the northern calcrete desert plains and the southern sand dunes) and the presence of local biological phenomena such the enigmatic fairy circles and the living plant-*fossil Welwitschia mirabilis* (Valverde et al., 2016, 2016van der Walt et al., 2016), make the entire region of considerable (micro)biological interest (Seely and Pallett, 2012). Past studies on the microbial ecology of the Namib Desert soils have focussed largely on the prokaryote microbiome (Cowan et al., 2020; Ramond et al., 2019), with the observation that the edaphic bacterial communities across the desert xeric gradient are structured by the effects of soil physicochemical parameters and xeric stress (Gombeer et al., 2015; Johnson et al., 2017; Scola et al., 2018). However, no comprehensive analyses of edaphic fungal diversity across the Namib Desert xeric gradient have yet been reported.

Fungi are ubiquitously present in the environment and can tolerate extremely diverse environmental conditions, including the desiccation and temperature extremes of deserts (Santiago et al., 2018; Selbmann et al., 2005). Arid soils are primarily dominated by the fungal phyla Ascomycota, Basidiomycota and Chytridiomycota (Fuentes et al., 2020; Maestre et al., 2015; Valverde et al., 2016, 2016van der Walt et al., 2016; Vargas-Gastélum et al., 2015; Zhang et al., 2013). Soil fungal communities generally show higher resistance to environmental disturbances than prokaryotic communities (Barnard et al., 2013; De Vries et al., 2012). Nevertheless, a number of climatic and edaphic variables have been reported to affect edaphic fungal diversity (Barnard et al., 2013; De Vries et al., 2012; ; Johnson et al., 2017; Maestre et al., 2015; Vargas-Gastélum et al., 2015). Increasing aridity has particularly shown to decrease soil fungal abundance and diversity (Jiao et al., 2021; Maestre et al., 2015). In the Namib Desert, edaphic fungal

* Corresponding author.
*E-mail address:* Don.cowan@up.ac.za (D.A. Cowan).

https://doi.org/10.1016/j.funbio.2023.03.001

communities are dominated by Ascomycota independently of the soil studied; i.e., dune vs gravel plain or rhizosphere/rhizosheath vs open soils (Marasco et al., 2018; Valverde et al., 2016, 2016van der Walt et al., 2016). A recent diel shotgun meta-transcriptomics analysis of low-rainfall Namib Desert gravel plain soils (León-Sobrino et al., 2019) also indicated that Ascomycota were the most active fungal taxa, particularly during the cooler night hours. Furthermore, edaphic fungal communities were found to assemble primarily through deterministic processes and particularly environmental filtering (Johnson et al., 2017), and to be strongly influenced by soil composition (particularly clay and sand content) and by soil $NH_4^+$ and total organic carbon contents (Johnson et al., 2017). However, these studies compared very different Namib Desert edaphic systems (i.e., dune vs gravel plain vs salt pans vs riverbeds and/or open soils vs plant-associated rhizosphere/rhizosheaths; Johnson et al., 2017; Marasco et al., 2018; Valverde et al., 2016; van der Walt et al., 2016) which can bias the results of such analyses (Ramsfield et al., 2020; Rao et al., 2016; Schreckinger et al., 2021; Weingarten et al., 2020).

Therefore, in order to understand the quantitative and qualitative nature of the spatial, geochemical and climatic factors that affect desert soil fungal communities, we evaluated fungal community diversity and assembly along a 220 km longitudinal xeric gradient through the central Namib Desert gravel plains (Scola et al., 2018). We hypothesized that, as for the prokaryote community (Frossard et al., 2015; Scola et al., 2018; Stomeo et al., 2013), deterministic niche partitioning, through historical climatic conditions and water-regime history (e.g., fog vs low precipitation vs high precipitation: Bosch et al., 2022) and local soil physicochemistry should be the dominant drivers of the fungal community assembly. However, given the high dispersal potential of fungal spores (Kellogg and Griffin, 2006), we also expected stochasticity to significantly influence Namib Desert gravel plain edaphic fungal communities.

## 2. Materials and methods

### 2.1. Sampling sites and sample collection

Soil samples were collected along a 200 km longitudinal transect across the Namib Desert in April 2017. Ten sampling sites, spaced at 20 km intervals (Fig. 1), traversed the three 'xeric' zones of the central Namib Desert, defined by their historical precipitation regimes (Eckardt et al., 2013; Hamilton and Seely, 1976; Lancaster et al., 1984). Within the transect, regular coastal fog events can reach 75 km inland and provide up to 183 mm mean annual precipitation (Eckardt et al., 2013; Lancaster et al., 1984). Furthermore, rain events are generally infrequent but increase from the coast towards inland areas, i.e., eastwards across the transect and from 0 to 250 mm mean annual precipitation (Eckardt et al., 2013). For consistency, the three west-to-east xeric zones are henceforth referred to as the 'fog' zone (sites 2 to 6), the 'low-rainfall' zone (sites 8 to 14) and the 'high-rainfall' zone (sites 16 to 20; Fig. 1; Scola et al., 2018). Data retrieved from SASSCAL weather stations (SASSCAL WeatherNet, 2020) indicate that, from April 2016 to April 2017, precipitation amounted to 3.4 mm, 58 mm and 119.2 mm in the 'fog' (Kleinberg [Station ID: 101], 'low-rainfall' (Garnet Koppie [Station ID: E7628]) and 'high-rainfall' (Rooisand [Station ID: 103]) zones, respectively. Mean annual temperatures were recorded as 18 °C, 22.2 °C and 24.6 °C in the three zones. At each site, four replicate (~500 g) surface soil (0–5 cm depth) samples were collected aseptically at 50m intervals along a 200m transect. Samples were transported at room temperature (~25–30 °C) to the University of Pretoria, then stored at −20 °C and 4 °C for downstream molecular and physicochemical analyses, respectively.

### 2.2. Soil physicochemical characterization

Soil physicochemical properties (pH, conductivity, phosphorous (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chloride (Cl), sulphate ($SO_4$), ammonium ($NH_4$) and nitrate ($NO_3$)) were analysed according to standard protocols by Bemlab (Pty) Ltd (Strand, Western Cape, South Africa). The Walkey-Black method (Walkley, 1935) was used to determine total organic carbon content. Soil structure was determined using the hydrometer method (Bouyoucos, 1962) at the Department of Plant and Soil Science, University of Pretoria, South Africa.

### 2.3. Soil DNA extraction and sequencing of the amplicon library

Metagenomic DNA was extracted from all soil samples using the PowerSoil® DNA Isolation Kit (MoBio Laboratories, CA, USA) according to the manufacturer's instructions. The fungal ITS intergenic region was amplified using the ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) primer set (White et al., 1990) in 25 μL reaction volume. An initial denaturation at 96 °C for 10 min was followed by 30 cycles of 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension step of one cycle at 72 °C for 10 min. Amplicon sequencing was performed according to the manufacturer's guidelines for the ITS1-2 region, using an Illumina MiSeq platform (MRDNA, Shallowater, TX, USA).

### 2.4. Sequence data analysis

Illumina MiSeq sequencing yielded a total of 3,969,628 sequences. Of the 40 amplicon libraries, two (i.e., C14.8. B and C14.8. D) failed to produce any sequencing output and were excluded from subsequent analyses. Barcoded sequence data were analysed using the QIIME2 v2020.8.0 platform (Bolyen et al., 2019). Default parameters were used to remove low-quality sequences during the Amplicon sequence variants (ASVs) clustering step. The DADA2 (Callahan et al., 2016) plugin for QIIME2 was used for chimera detection and clustering of ASVs. Quality filtering parameters were selected from demultiplexed data; 31 for trim-left and 204 for trunc-len (truncation length); using the DADA2 denoise-single method for trimming low-quality regions of the sequences. Fungal taxonomy was assigned to representative ITS gene sequences using the QIIME2 feature-classifier *classify-sklearn* and the UNITE database (Abarenkov et al., 2010). Alpha diversity indices were analysed in QIIME2.

The ITS sequencing reads were submitted to the NCBI SRA database under the Bioproject accession number PRJNA890764 and SRA accession IDs from SRR21912621 to SRR21912658.

### 2.5. Statistical analyses

Statistical analyses were primarily performed in R version 3.6.0 (R Core Team, 2019). The recorded values for the environmental factors were normalised using the "log" method in "decostand" function to create a correlation-based principal component analysis (PCA) in Vegan package (Oksanen et al., 2013). The significant differences between the xeric transect zone environmental variables were analysed using ANOVA followed by Kruskal–Wallis and Benjamini-Hochberg (BH) *p*-value corrections.

Fungal taxonomy bar plots were created in the Phyloseq package (McMurdie and Holmes, 2013) in R (R Core Team, 2019). Principal coordinate analysis (PCoA) plots were produced using the rarefied, Hellinger-transformed data with Bray–Curtis distances and visualised using the Phyloseq package. Co-occurrence null model analysis was performed using the iCAMP package (Ning et al., 2020)

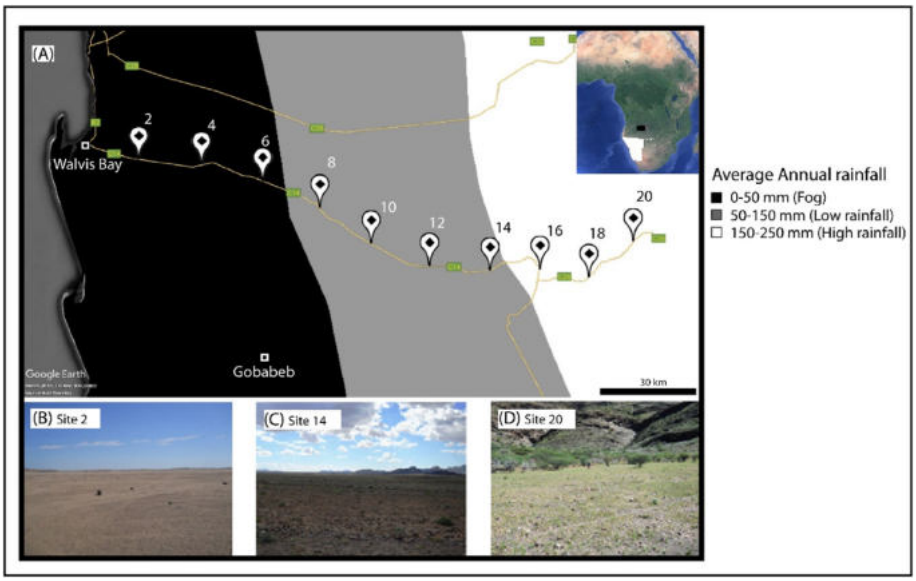


**Fig. 1.** **(A)** Average annual rainfall of the transect. Rainfall gradually increases across the transect to the interior of the country **(B)** Fog zone, bare ground, sample sites 2–6 **(C)** Low rainfall zone with little vegetation, sample sites 8–14 **(D)** High rainfall zone with higher vegetative biomass, sample sites 16–20.

in R. Phylogenetic trees were constructed using the taxonomy_to_tree.pl script available from a previously published method (Tedersoo et al., 2018). The analysis was performed using the beta net relatedness index (βNRI) and modified Raup-Crick metric (RC). The iCAMP analysis was performed using the phylogenetic binning and threshold cut-off for $\beta NRI < -1.96$ for homogeneous selection, and $\beta NRI > +1.96$ for heterogeneous selection. Taxonomic diversity metrics were used for partitioning the remaining pairwise comparisons with $|\beta NRI| \leq 1.96$. $RC < -0.95$ was used for homogenising dispersal and $RC > +0.95$ for dispersal limitation. Residuals with $|\beta NRI| \leq 1.96$ and $|RC| \leq 0.95$ represent the percentage of drift. The relative importance of each ecological process (Determinism: Heterogenous selection and Homogenous selection; Stochasticity: Homogenising dispersal, Dispersal limitation and Drift) was calculated in the iCAMP package using the "icamp.bins" function (Ning et al., 2020).

Tests of intra-group dispersal (Betadisper) and PERMANOVA, distance-based redundancy analysis (db-RDA) and variation partitioning were performed using the vegan package (Oksanen et al., 2013) in R (R Core Team, 2019). Environmental and spatial variables were transformed by applying the decostand function "method = log". The categorical values for the spatial variables (fog, low-rainfall and high-rainfall) were converted to the dummy values; 1 for presence and 0 for absence. Distance-based (db)-RDA analysis was performed using the ASV matrix and by applying forward selection of the environmental and spatial variables.

## 3. Results and discussion

In global scale soil studies, aridity has been identified as a key factor in controlling soil fertility and plant cover and productivity, along with the diversity and biomass of soil fungal and bacterial communities (Berdugo et al., 2020; Delgado-Baquerizo et al., 2013; Maestre et al., 2015). Fungal communities are important biological components of (desert) edaphic environments and are particularly critical for carbon turnover (Jastrow et al., 2007). However, globally, fungi are relatively understudied in hot desert soil ecosystems, compared to prokaryotes (Cowan et al., 2020).

Previous studies have suggested that in many Namib Desert edaphic niches, bacterial, archaeal (Frossard et al., 2015; Gombeer et al., 2015; Johnson et al., 2017; Scola et al., 2018, 2018van der Walt et al., 2016) and fungal (Johnson et al., 2017, 2017van der Walt et al., 2016) communities are structured by deterministic processes and particularly by environmental niche filtration. Identified edaphic physicochemical parameters such as phosphorus, sodium, sulphur, percent sand and clay were found to shape the fungal communities in the Namib Desert dune sands and gravel plain soils (Johnson et al., 2017, 2017van der Walt et al., 2016). However, almost all these studies compared fungal communities from very contrasted edaphic systems (i.e., dune vs gravel plain vs saltpans vs riverbed) using molecular fingerprinting methods. As a result, to more specifically understand the drivers of fungal community assembly in deserts, we studied the mycobiome along an east-west transect within a single and dominant soil terrain type, the Namib Desert gravel plains, using metabarcoding.

### 3.1. Soil physicochemical parameters vary across the Namib Desert gravel plains

As observed in previous studies (Johnson et al., 2017; Ramond et al., 2018; Scola et al., 2018), some soil physicochemical properties were significantly different within the three xeric zones of the transect (PERMANOVA Global Test and Pairwise comparisons, $p < 0.05$; Table 1; Fig. 2; Supplementary Fig. 1). The fog, low-rainfall and high-rainfall zone samples were characterized by higher ionic compound, ammonium and carbon contents, respectively (Supplementary Table 1). The high ionic concentrations in fog zone soil samples have been attributed to the transport and deposition of marine aerosols into the coastal region of the transect (Gustafsson and Franzén, 1996; Liang et al., 2016) rather than the effect of fog precipitation. The higher phosphorus (P) and carbon (C) contents and higher pH values in the high-rainfall zone are attributed to the relatively higher primary productivity compared to the other two zones (Ramond et al., 2018; Scola et al., 2018). These data are consistent with the concept that both historical water regime histories (e.g., fog vs rain) and proximity to the ocean influence local soil physicochemistries.

**Table 1**
PERMANOVA results showing differences in physicochemical and biological characteristics between the xeric zones. Df = degree of freedom; F= Psuedo F; P = p value. *: Significant results ($p < 0.05$).

| Environmental variable and fugal community | PERMANOVA | | |
|---|---|---|---|
| | df | F | P |
| Global Test | 2 | 8.4341 | 0.001* |
| Fog-low rainfall | 1 | 10.5625 | 0.001* |
| Fog-high rainfall | 1 | 11.4074 | 0.001* |
| Low rainfall-high rainfall | 1 | 3.2878 | 0.001* |
| **Fungal Communities only** | | | |
| Global test | 2 | 7.6443 | 0.001* |
| Fog-low rainfall | 1 | 9.2798 | 0.001* |
| Fog-high rainfall | 1 | 11.315 | 0.001* |
| Low rainfall-high rainfall | 1 | 2.61 | 0.001* |

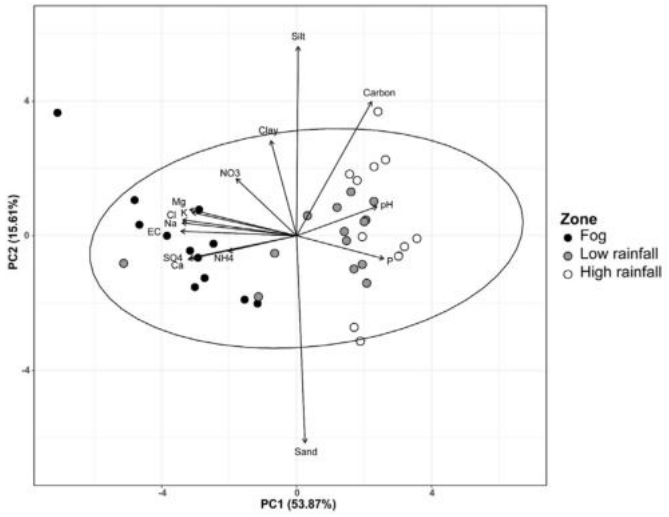


**Fig. 2. Principal component analysis plot of the soil physicochemical parameters.** The samples were clustered based on the correlation of environmental and physicochemical variables on the first two Axes of the PCA plot.

### 3.2. Taxonomic composition of the central Namib Desert gravel plain fungal communities

The sequence clustering of Amplicon Sequence Variants (ASVs), from a total of 3,732,846 sequences, yielded sets of 19,059 to 445,529 sequences for the 38 samples. Rarefaction analysis with 2508 ASVs (19,059 sequences for any sample) clearly showed adequate sequence coverage of fungal diversity (Supplementary Fig. 2). The fungal communities in the three zone groups were found to be compositionally different (PERMANOVA Global test *pseudo-F* = 7.6443, $p = 0.001$ and Pairwise comparisons $p < 0.05$; Table 1) and globally very diverse; i.e., composed of 7 phyla, 23 classes, 48 orders, 95 families, 158 genera and 162 species. We note the high proportion of unidentified fungal taxa (31% on average but >75% at some sampling sites [e.g., C14–4B and C14-8A]; Fig. 3A; Supplementary Fig. 3), supporting the contention that, along with other microeukaryotic and viral communities (Cowan et al., 2020), fungi remain poorly characterized in arid environments.

As previously shown (Marasco et al., 2018; Valverde et al., 2016, 2016van der Walt et al., 2016), three phyla, namely, Ascomycota (41%), Basidiomycota (18%) and Chytridiomycota (9%) (Fig. 3A), clearly dominated the Namib Desert edaphic mycobiome. The overall dominance of members of the phylum Ascomycota and the low relative abundance of Chytridiomycota in arid environment mycobiomes has also been reported (Jacobson et al., 2015; Jiao et al., 2021; León-Sobrino et al., 2019; Murgia et al., 2019; Santiago et al., 2018; Sterflinger et al., 2012, 2012van der Walt et al., 2016; Yang et al., 2017). Chytridiomycota represented 14% and 10% of the low-rainfall and high-rainfall soil sample communities, respectively (Fig. 3A), but were not detected in fog-zone communities. Similarly, Ascomycota were significantly more abundant in the high-rainfall zone than in fog zone communities (Tuckey's HSD, $q = 0.18$; Supplementary Table 2). In contrast, Basidiomycota abundances did not differ significantly across the three zones (ANOVA $p > 0.05$). Other fungal phyla (Rozellomycota, Mucoromycota, Mortierellomycota, and Calcarisporiellomycota) were identified at less than 2% relative abundance and showed no significant differences between the xeric zones.

At the genus level, fog zone soils were significantly enriched in *Cladosporium* (Ascomycota; fog-low rainfall, $q = 0.005$; fog-high rainfall, $q = 0.005$), *Malassezia* (Basidiomycota; fog-low rainfall, $q = 0.003$; fog-high rainfall, $q = 0.004$) and *Alternaria* (Ascomycota; fog-low rainfall, $q = 0.033$; fog-high rainfall, $q = 0.038$) and significantly depleted in members of the *Monosporascus* (Ascomycota; fog-low rainfall, $q = 0.036$) and *Rhizophlyctis* (Chytridiomycota; fog-low rainfall, $q = 0.0004$; fog-high rainfall, $q = 0.035$) genera (Supplementary Table 2). The higher relative abundance of the genera *Cladosporium*, *Malassezia* and *Alternaria* in the fog zone parallels observations from the Atacama and Namib Desert (Santiago et al., 2018, 2018van der Walt et al., 2016), suggesting that the resident members of these fungal genera may be specifically adapted to low water-activity and higher soil salinity habitats (Sepcic et al., 2010). *Westerdykella* were significantly more abundant in high-rainfall zone soils (Ascomycota, $q < 0.05$; Supplementary Table 2).

Of the 2508 fungal ASVs identified, 73.2% (i.e., 1835/2508) were zone-specific; i.e., exclusively present in either the fog (156/2508; 6.2%), low-rainfall (557/2508; 22.2%) or high-rainfall (1122/2508; 44.7%) zone soils (Supplementary Fig. 4). 600 fungal ASVs were shared between the low-rainfall and high-rainfall zones, while very few were shared between the other two zone pairs (fog/low-rainfall [57] and fog/high-rainfall [1]; Supplementary Fig. 4).

Altogether, these results strongly suggest that environmental filtering participates in fungal community assembly along the Namib Desert gravel plains transect. The abundance data also suggest that the dominant transition in community composition occurs at the limit of fog influence. It is noted that edaphic prokaryotic and viral communities showed a similar trend (Scola et al., 2018). We therefore suggest that the inland limit of fog penetration, and therefore water regime history and its influence of soil chemistry (Frossard et al., 2015; Supplementary Fig. 1, Supplementary Table 1), represents an ecological barrier for fungal dispersal in the Namib Desert (Shapiro and Polz, 2014).

The core mycobiome of the Namib Desert gravel plains; i.e., the fungal taxa common to all three zones, was only composed of fifteen ASVs (0.6% of the total ASVs but representing 6.7% of total rarefied sequences). This further confirms our contention that intense selective pressure occurs in the different xeric zone soils; i.e., deterministic niche-partitioning (Nemergut et al., 2013). All members of the core mycobiome were assigned to the Ascomycota Dothideomycetes (orders Pleosporales, Capnodiales, Dothideales and Hypocreales) and Basidiomycota Tremellomycetes (order Filobasidiales) classes (Supplementary Table 3). Dothideomycetes are known to be able to tolerate multiple environmental extremes - including those typical of hot deserts (i.e., high temperatures, high solar radiation fluxes and extreme dehydration; Murgia et al., 2019), and Filobasidiales are dominant fungal taxa in Atacama Desert soils (Schmidt et al., 2012).

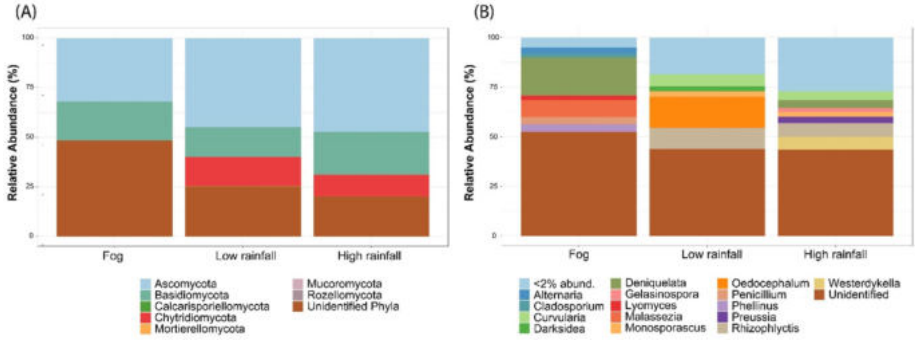


**Fig. 3. The relative abundance of fungal taxa in Namib xeric gradient soils (A)** The relative abundance of phylum in fog, low-rainfall and high-rainfall zone soil **(B)** Relative abundance of the fungal genus in fog, low-rainfall and high-rainfall zone soil. Fungal genera representing less than 2% of relative abundance were collectively annotated as "<2% abundance".

### *3.3. Namib Desert edaphic fungal community assembly is dominated by stochastic processes*

The Namib Desert gravel plain fungal communities significantly differed in the three longitudinal xeric zones as their α-diversity, evenness and Shannon indices values were significantly distinct and increased from the coast towards inland areas (Fig. 4). This is also evident in the clustering of each samples according to their xeric zone of origin in PCoA and RDA plots (Fig. 5). RDA analysis, which explained 64.8% of fungal community variance, identified climatic (fog; $p = 0.002$) and various edaphic variables ($SO_4$, Mg, P, Ca and Silt; $p < 0.05$) linked to the deposition of marine aerosols (Gustafsson and Franzén, 1996; Liang et al., 2016) as abiotic drivers of fungal community structures (Fig. 5B, Supplementary Table 4). Altogether, these results further support the conclusion that environmental niche partitioning is a significant driver of fungal community assembly in Namib Desert gravel plain soils and that the coastal edaphic environment has higher selectivity (Maestre et al., 2015; Ramond et al., 2014).

However, variation partitioning analyses showed that only 20% of the variation of fungal community assembly along the xeric transect was explained by the 15 soil physicochemical and 3 spatial (xeric zonation) parameters; 16% and 4%, respectively (Table 2; Supplementary Fig. 5). The high proportion of the unexplained variations combined with the low contribution of soil physicochemical and the spatial variables suggest that stochasticity, species interactions, neutral processes and other unmeasured soil physicochemical and environmental variables probably contribute to fungal edaphic community assembly in the Namib Desert (Gunnigle et al., 2017; Ladau and Eloe-Fadrosh, 2019; Nemergut et al., 2013). Niche-based assembly processes have been found to dominate Namib Desert edaphic soil fungal community assembly when comparing various edaphic niches (Johnson et al., 2017). However, and contrastingly, at the global scale, edaphic fungal communities were found to assemble by stochastic rather than deterministic processes (Powell and Bennett, 2016).

Phylogenetic-bin-based null model analysis (iCAMP; Ning et al., 2020) confirmed that Namib Desert gravel plain fungal communities assemble via a combination of deterministic and stochastic processes but that the latter clearly dominated within all three xeric zones (ranging from 80.8% to 94.3%; Supplementary Fig. 6), as observed in global studies (Powell and Bennett, 2016). More specifically, dispersal limitation increased from the fog (49.9%) to the low-rainfall zone (72.2%) – i.e., from the inland from the coast - and drift was lowest in the rainfall zones (21.6% vs 31% and 30.4% in the low-rainfall and the fog zones, respectively). This suggests that

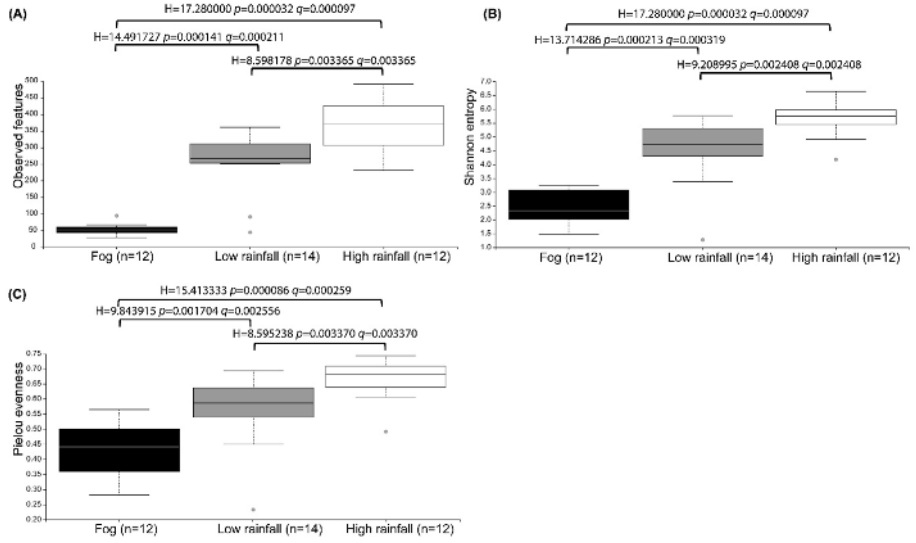


**Fig. 4. Alpha diversity index boxplots using (A) Observed ASVs (B) Shannon entropy and (C) Evenness.** Pairwise comparison outputs are written on the top of the bar-plots. A Kruskal–Wallis's test followed by FDR correction was applied to identify the significant differences between the alpha diversity indices. A corrected *p-value*, $q \leq 0.05$ used for the significance cut-off.

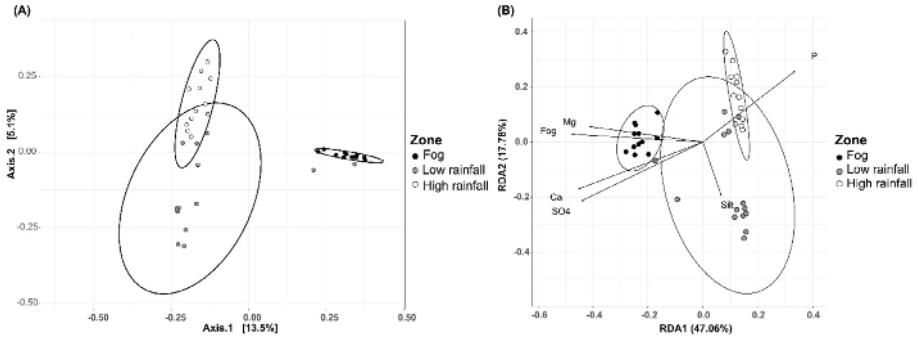


**Fig. 5. Principal coordinate analysis (PCoA) plot and distance-based redundancy analysis (db-RDA) plot. A)** The rarefied ASV table was Hellinger transformed and Bray–Curtis distances were plotted using PCoA. **B)** db-RDA plot showing the significant soil physiochemical and spatial parameters. The cut-off for significance was $p < 0.05$.

**Table 2**
Variation portioning of the structure of fungal communities into soil physicochemical [P] and spatial [S] and overlap between these components [PS] using redundancy analysis.

| | |
|---|---|
| Total explained variation | 0.191 |
| Soil physicochemical variables [P] | 0.045 |
| Spatial [S] | −0.0008 |
| Overlap of P and S [PS] | 0.146 |
| Unexplained variation | 0.809 |
| *P* value | 0.007 |

Namib Desert edaphic fungal communities are sensitive to local environmental conditions and that ecological barriers affect their dispersal (Shapiro and Polz, 2014). The fact that deterministic processes were highest (19.2%; Supplementary Fig. 6) for the fog zone community and that this community exhibited the lowest taxonomic diversity (Fig. 4) clearly suggests that soils from this zone exert a stronger selective pressure than those from other xeric zones. This effect may be linked to the higher salt content of the fog zone soils (Table 1, Fig. 5B; Scola et al., 2018).

As different members from any microbial community may be under the influence of different selective pressures (Nemergut et al., 2013; Ning et al., 2020), we further evaluated the influence of each assembly mechanism on fungal community lineages within each xeric zone (Supplementary Table 5; Ning et al., 2020). The 2508 fungal ASVs were grouped into 47 bins; i.e., 3, 10 and 32 of the Chytridiomycota, Basidiomycota and Ascomycota fungal phyla, respectively, as well as 2 from unidentified fungal phyla (Supplementary Table 5). Supporting community level analyses, the majority of the fungal lineages were influenced by stochastic processes, particularly drift (representing 61.5% [16/26], 42.6% [20/47] and 11.1% [5/45] of bin numbers in the fog, low-rainfall and high-rainfall zones, respectively) and dispersal limitation (26.9% [7/26], 51.1% [24/47] and 86.7% [39/45]). Only two bins related to the fungal species *Oedocephalum adhaerens* [Basidiomycota] and *Ophiostoma rachisporum* [Ascomycota] showed a statistically significant ($p < 0.05$) dominant deterministic assembly process, specifically homogenous selection. Both were derived from fog zone samples. The presence of these fungi in desert soils may be linked to their host-associations: the former has been isolated from animal dung (Ghosta et al., 2016) and the latter is insect-associated (e.g., Jankowiak et al., 2017).

## 4. Conclusions

This study represents the first comprehensive metagenomic analysis of fungal diversity across a desert xeric gradient, providing an opportunity to clarify the competing drivers of species distribution: homogeneous distribution resulting from stochastic processes such as aerial transport of spores, versus deterministic processes driven by abiotic variables such as water availability and soil salinity. For convenience, we used an established longitudinal zonation (Bosch et al., 2022; Scola et al., 2018).

The observation that a high proportion of fungal ASVs (approx. 73%) were zone-specific, and that very few ASVs (0.6%) were common to all zones (the core mycobiome), are both strong indicators that environmental filtering is a dominant driver of species distribution. However, the fractional contribution of soil properties and xeric status suggests that other, undetermined, variables substantially impact community composition. We suggest that biotic factors, particularly inter-species interactions, may play a significant role (Gunnigle et al., 2017). However, we also note that the distribution of fungal taxa across the desert xeric gradient may also be somewhat dissociated from local environmental conditions (i.e., historical climatic regime and soil physicochemistries) due to aeolian transport and widespread distribution of fungal spores (Nemergut et al., 2013). Phylogenetic distribution data indicate that the coastal fog zone imposes the strongest selection on edaphic fungal communities. The unusual water relations (Bosch et al., 2022) and the elevated salinity of soils in this zone provide a credible deterministic mechanism for fungal community composition in coastal soils of the Namib Desert.

The very high proportion of unidentified fungal ASVs in some samples suggests that Namib Desert soils retain a substantial residue of novel taxa, and argues strongly for more in-depth phylogeographical surveys of the region, preferably supported by culture-based studies.

## Authors' contributions

DAC funded and supervised the project. KP, J-BR and DAC designed the experiment. MSc student KP performed the experimental work. Data analyses were performed by SV, KP and MO. GMK provided logistical support in the Namib Desert. SV, DAC and J-BR wrote the manuscript. All authors read and approved the final draft of the manuscript.

## Acknowledgement

The authors gratefully acknowledge financial support from the National Research Foundation of South Africa and the University of Pretoria.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2023.03.001.

## References


Abarenkov, K., Nilsson, R.H., Larsson, K.-H., et al., 2010. The UNITE database for molecular identification of fungi–recent updates and future perspectives. New Phytol. 186, 281–285.

Barnard, Rl, Osborne, C.A., Firestone, M.K., 2013. Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. ISME J. 7, 2229–2241.

Berdugo, M., Delgado-Baquerizo, M., Soliveres, S., et al., 2020. Global ecosystem thresholds driven by aridity. Science 367, 787–790.

Bolyen, E., Rideout, J.R., Dillon, M.R., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.

Bosch, J., Marais, E., Maggs-Kölling, G., Ramond, J.-B., Lebre, P.H., Eckardt, F., Cowan, D.A., 2022. Water inputs across the Namib Desert: implications for dryland edaphic microbiology. Frontiers in Biogeography 14, e55302.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analyses of soils 1. Agron. J. 54, 464–465.

Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., et al., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.

Cowan, D.A., Hopkins, D.W., Jones, B.E., et al., 2020. Microbiomics of Namib Desert habitats. Extremophiles 24, 17–29.

De Vries, F.T., Liiri, M.E., Bjørnlund, L., et al., 2012. Land use alters the resistance and resilience of soil food webs to drought. Nat. Clim. Change 2, 276–280.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., et al., 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502, 672–676.

Eckardt, F., Soderberg, K., Coop, L., et al., 2013. The nature of moisture at Gobabeb, in the central Namib Desert. J. Arid Environ. 93, 7–19.

Frossard, A., Ramond, J.-B., Seely, M., Cowan, D.A., 2015. Water regime history drives responses of soil Namib Desert microbial communities to wetting events. Sci. Rep. 5, 12263.

Fuentes, A., Herrera, H., Charles, T.C., Arriagada, C., 2020. Fungal and bacterial microbiome associated with the rhizosphere of native plants from the Atacama desert. Microorganisms 8, 209.

Ghosta, Y., Poursafar, A., Qarachal, J.F., 2016. Study on coprophilous fungi: new records for Iran mycobiota. Rostaniha 17, 115–126.

Gombeer, S., Ramond, J.-B., Eckardt, F., et al., 2015. The influence of surface soil physicochemistry on the edaphic bacterial communities in contrasting terrain types of the Central Namib Desert. Geobiology 13, 494–505.

Gunnigle, E., Frossard, A., Ramond, J.-B., Guerrero, L., Seely, M., Cowan, D.A., 2017. Diel-scale temporal dynamics recorded for bacterial groups in Namib Desert soil. Sci. Rep. 7, 40189.

Gustafsson, M.E., Franzén, L.G., 1996. Dry deposition and concentration of marine aerosols in a coastal area, SW Sweden. Atmos. Environ. 30, 977–989.

Hamilton, W.J., Seely, M.K., 1976. Fog basking by the Namib Desert beetle, *Onymacris unguicularis*. Nature 262, 284–285.

Jacobson, K., Van Diepeningen, A., Evans, S., et al., 2015. Non-rainfall moisture activates fungal decomposition of surface litter in the Namib Sand Sea. PLoS One 10, e0126977.

Jankowiak, R., Strzałka, B., Bilański, P., et al., 2017. Page diversity of ophiostomatales species associated with conifer-infesting beetles in the western carpathians. Eur. J. For. Res. 136, 939–956.

Jastrow, J.D., Amonette, J.E., Bailey, V.L., 2007. Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Climatic Change 80, 5–23.

Jiao, S., Zhang, B., Zhang, G., et al., 2021. Stochastic community assembly decreases soil fungal richness in arid ecosystems. Mol. Ecol. 30, 4338–4348.

Johnson, R.M., Ramond, J.B., Gunnigle, E., et al., 2017. Namib Desert edaphic bacterial, fungal and archaeal communities assemble through deterministic processes but are influenced by different abiotic parameters. Extremophiles 21, 381–392.

Kellogg, C.A., Griffin, D.W., 2006. Aerobiology and the global transport of desert dust. Trends Ecol. Evol. 21, 638–644.

Ladau, J., Eloe-Fadrosh, E.A., 2019. Spatial, temporal, and phylogenetic scales of microbial ecology. Trends Microbiol. 27, 662–669.

Lancaster, J., Lancaster, N., Seely, M., 1984. Climate of the central Namib Desert. Madoqua 14, 5–61.

León-Sobrino, C., Ramond, J.-B., Maggs-Kölling, G., Cowan, D.A., 2019. Nutrient acquisition, rather than stress response over diel cycles, drives microbial transcription in a hyper-arid Namib Desert soil. Front. Microbiol. 10, 1054.

Liang, T., Chamecki, M., Yu, X., 2016. Sea salt aerosol deposition in the coastal zone: a large eddy simulation study. Atmos. Res. 180, 119–127.

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., et al., 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences,* USA 112, 15684–15689.

Marasco, R., Mosqueira, M.J., Fusi, M., et al., 2018. Rhizosheath microbial community assembly of sympatric desert speargrasses is independent of the plant host. Microbiome 6, 215.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217.

Murgia, M., Fiamma, M., Barac, A., et al., 2019. Biodiversity of fungi in hot desert sands. Microbiology Open 8, e00595.

Nemergut, D.R., Schmidt, S.K., Fukami, T., et al., 2013. Patterns and processes of microbial community assembly. Microbiol. Mol. Biol. Rev. 77, 342–356.

Ning, D., Yuan, M., Wu, L., et al., 2020. A quantitative framework reveals ecological drivers of grassland microbial community assembly in response to warming. Nat. Commun. 11, 4717.

Oksanen, J., Blanchet, F.G., Kindt, R., et al., 2013. Package 'vegan', 2. Community Ecology Package, version, pp. 1–295.

Powell, J.R., Bennett, A.E., 2016. Unpredictable assembly of arbuscular mycorrhizal fungal communities. Pedobiologia 59, 11–15.

R CORE TEAM, 2019. R: A Language and Environment for Statistical Computing.

Ramond, J.-B., Woodborne, S., Hall, G., et al., 2018. Namib Desert primary productivity is driven by cryptic microbial community N-fixation. Sci. Rep. 8, 1–9.

Ramond, J.-B., Baxter, J., Maggs-Kölling, G., et al., 2019. Microbial ecology of the Namib Desert. In: Seckbach, J., Rampelotto, P. (Eds.), Model Ecosystems in Extreme Environments. Academic Publ, pp. 113–143 (Chapter 6).

Ramond, J.-B., Pienaar, A., Armstrong, A., Seely, M., Cowan, D.A., 2014. Niche-partitioning of edaphic microbial communities in the Namib Desert gravel plain Fairy Circles. PLoS One 9, e109539.

Ramsfield, T., Shay, P.-E., Trofymow, T., et al., 2020. Distance from the forest edge influences soil fungal communities colonizing a reclaimed soil borrow site in boreal mixedwood forest. Forests 11, 427.

Rao, S., Chan, Y., Bugler-Lacap, D.C., et al., 2016. Microbial diversity in soil, sand dune and rock substrates of the Thar Monsoon Desert, India. Indian J. Microbiol. 56, 35–45.

Santiago, I.F., Gonçalves, V.N., Gómez-Silva, B., et al., 2018. Fungal diversity in the Atacama Desert. Antonie Leeuwenhoek 111, 1345–1360.

SASSCAL WeatherNet, 2020. www.sasscalweathernet.org.

Schmidt, S., Naff, C., Lynch, R., 2012. Fungal communities at the edge: ecological lessons from high alpine fungi. Fungal Ecology 5, 443–452.

Schreckinger, J., Mutz, M., Mendoza-Lera, C., et al., 2021. Attributes of drying define the structure and functioning of microbial communities in temperate riverbed sediment. Front. Microbiol. 12, 1537.

Scola, V., Ramond, J.B., Frossard, A., et al., 2018. Namib Desert Soil microbial community diversity, assembly, and function along a natural xeric gradient. Microb. Ecol. 75, 193–203.

Seely, M., Pallett, J., 2012. Namib: Secrets of a Desert Uncovered. Venture Publications.

Selbmann, L., De Hoog, G., Mazzaglia, A., et al., 2005. Fungi at the edge of life: cryptoendolithic black fungi from Antarctic desert. Stud. Mycol. 51, 1–32.

Sepcic, K., Zalar, P., Gunde-Cimerman, N., 2010. Low water activity induces the production of bioactive metabolites in halophilic and halotolerant fungi. Mar. Drugs 9, 43–58.

Shapiro, B.J., Polz, M.F., 2014. Ordering microbial diversity into ecologically and genetically cohesive units. Trends Microbiol. 22, 235–247.

Sterflinger, K., Tesei, D., Zakharova, K., 2012. Fungi in hot and cold deserts with particular reference to microcolonial fungi. Fungal Ecology 5, 453–462.

Stomeo, F., Valverde, A., Pointing, S.B., et al., 2013. Hypolithic and soil microbial community assembly along an aridity gradient in the Namib Desert. Extremophiles 17, 329–337.

Valverde, A., De Maayer, P., Oberholster, T., et al., 2016. Specific microbial communities associate with the rhizosphere of *Welwitschia mirabilis*, a living fossil. PLoS One 11, e0153353.

Van Der Walt, A.J., Johnson, R.M., Cowan, D.A., et al., 2016. Unique microbial phylotypes in Namib Desert dune and gravel plain Fairy Circle soils. Appl. Environ. Microbiol. 82, 4592–4601.

Vargas-Gastélum, L., Romero-Olivares, A.L., Escalante, A.E., et al., 2015. Impact of seasonal changes on fungal diversity of a semi-arid ecosystem revealed by 454 pyrosequencing. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol. 91, fiv044.

Walkley, A., 1935. An examination of methods for determining organic carbon and nitrogen in soils. J. Agric. Sci. 25, 598–609.

Weingarten, E.A., Lawson, L.A., Jackson, C.R., 2020. The saltpan microbiome is structured by sediment depth and minimally influenced by variable hydration. Microorganisms 8, 538.

White, T.J., Bruns, T., Lee, S., et al., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–322.

Yang, T., Adams, J.M., Shi, Y., et al., 2017. Fungal community assemblages in a high elevation desert environment: absence of dispersal limitation and edaphic effects in surface soil. Soil Biol. Biochem. 115, 393–402.

Zhang, X., Zhao, L., Xu, J.R., et al., 2013. Soil moisture effect on bacterial and fungal community in Beilu River (Tibetan Plateau) permafrost soils with different vegetation types. J. Appl. Microbiol. 114, 1054–1065.