Title: Unique microbial phylotypes in Namib Desert dune and gravel plain Fairy Circle soils

Running title: Namib Desert Fairy Circles Microbial Communities

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Abstract

Fairy Circles (FCs) are barren circular patches of soil surrounded by grass species, the origin of which is poorly understood. FCs feature in both the gravel plains and dune fields of the Namib Desert. While a substantial number of hypotheses to explain the origin and/or sustainability of fairy circles have been presented, none are completely consistent with either their properties or distribution. In this study, we investigated the hypothesis that dune and gravel plain FC formation is due to microbial phytopathogenesis. Surface soils from five gravel plain and five dune FCs, together with control soil samples, were analysed using high-throughput sequencing of bacterial/archaeal (16S rRNA gene) and fungal (ITS region) phylogenetic markers. Our analyses showed that gravel plain and dune FC microbial communities are phylogenetically distinct and that FC communities differ from adjacent vegetated soils. Furthermore, various soil physicochemical properties, particularly pH, Ca, P, Na, SO_4, soil particle size and % carbon, significantly influenced dune and gravel plain FC microbial community compositions but none were found to segregate FC and vegetated soil communities. Nevertheless, 9 bacterial, 1 archaeal and 57 fungal phylotypes were identified as FC-specific, being present only within the gravel plain and dune FCs soils but not in the vegetated soils. Some of these FC-specific phylotypes were assigned to taxa known to harbour phytopathogenic microorganisms. This suggests that these FC-specific microbial taxa may be involved in the formation and/or maintenance of Namib Desert FCs.

Importance

Fairy Circles (FCs) are mysterious barren circular patches of soil found within a grass matrix in the dune fields and the gravel plains of the Namib Desert. Various hypotheses

attempting to explain this phenomenon have been proposed. However, to date, none have been successful in fully explaining their etiology; particularly as gravel plain FCs have been largely ignored. In this study, we investigated the hypothesis that microorganisms could be involved in the FC phenomenon through phytopathogenesis. We show that FC and control vegetated soil microbial communities were significantly different. Furthermore, Namib Desert edaphic bacterial and archaeal communities were found to assemble mainly through stochastic processes, whereas fungal communities rather through deterministic processes. Finally, we detected 67 FC-specific microbial phylotypes, i.e. solely present in both the gravel plain and the dune FC soils; some of which were closely related to known phytopathogens. Our results, therefore, demonstrate that microorganisms may play a role in the formation and/or maintenance of Namib Desert FCs, possibly *via* phytopathogenic activities.

Introduction

Fairy Circles (FCs) are circular patches of soil completely devoid of vegetation within a matrix of grass, typically *Stipagrostis* spp. (1, 2). Fairy Circles, which are common in the Namib Desert and have been recently observed in the Australian outback (3), are limited to arid areas which receive between 50 and 100 mm precipitation per annum. FCs are non-permanent, as they typically appear, enlarge and disappear with an estimated lifespan ranging from 22 to 60 years (4, 5).

Although FCs were first reported in the 1970's (6), there is currently no scientific consensus on the mechanisms behind their formation and maintenance (e.g., (2, 7-9)).

Faunal-based hypotheses suggest that FCs result from termite-, ant- or rodent-harvesting activities (1, 5, 10-12). The plant spatial self-organization hypothesis argues that FCs are the product of extreme aridity (13, 14). During times of drought, competition between plants for nutrients and water resources leads to the formation of barren patches (14), which in turn act as water traps, utilized by the peripheral grasses through extensive root systems and soil-water diffusion (13). Other published hypotheses implicate localized radioactivity hot spots (2), natural gas seepages (8), and the release of allelopathic compounds by dead *Euphorbia damarana* plants (5, 15). However, all previous studies testing these hypotheses have focussed solely on FCs located in the Namib Desert dunes (2, 4, 8, 10, 14, 15) while FCs also occur, albeit more sparsely, in the Namib Desert gravel plains (16). The latter distribution contradicts several of the extant hypotheses. For example, well-defined spatial patterning is not a characteristic of the gravel plain FCs, and *E. damarana* is not endemic to these regions. A recent hypothesis (16) has suggested the involvement of microorganisms in the FC phenomenon as, within gravel plain FCs, two fungal T-RFs were detected within FCs. However, their taxonomic identity could not be assigned due to the nature of the fingerprinting technique used (16). The fact that FCs are clear circular patterns with a distinct life cycle (2, 4) is compatible with the presence of a microbial phytotoxic compound which inhibits grass/plant growth (17). Although contested by recent *in situ* soil transfer experiments of 5 dune FCs (7), the phytotoxicity of FC soils has been shown in greenhouse experiments (2), an observation which is consistent with the presence of an active, possibly microbial, plant-inhibitory compound.

To further evaluate the involvement of microorganisms in FCs, we therefore investigated the edaphic microbial communities from both dune and gravel plain FCs, together with

control communities from adjacent vegetated soils. This is the first study comparing both the dune and gravel plain FCs. We used Illumina MiSeq high-throughput sequencing of the 16S rRNA gene and the ITS region to target the bacterial, archaeal and fungal members of each set of soil samples. Our aims were (i) to assess differences in microbial community structures between Namib Desert dune and gravel plain FC soils and control vegetated soils and (ii) to identify microorganisms solely present within dune and gravel plain FCs soils. The latter would constitute potential candidates for involvement in the formation and/or maintenance of Namib Desert FCs.

Materials and Methods

Sample collection

Namib Desert gravel plain (23°33′0″S, 15°2′0″E) and dune (24°32′47″S 15°19′47″E) soil samples were collected in April 2014. At each site, three pseudoreplicates (~400g) surface soils (0-5cm depth) were aseptically collected from the centre of 5 individual FCs and adjacent vegetated matrix sites (2 x radius from FC centre; *n* = 20; Figure 1). Samples were transported and stored at -20°C until further analyses.

Soil physicochemical analyses

For each soil sample, the three pseudoreplicates were combined in equal amounts (~50 g) and sieved (2 mm) prior to analysis. The soil pH, conductivity, sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chloride (Cl), sulphate (SO_4), phosphorous (P),

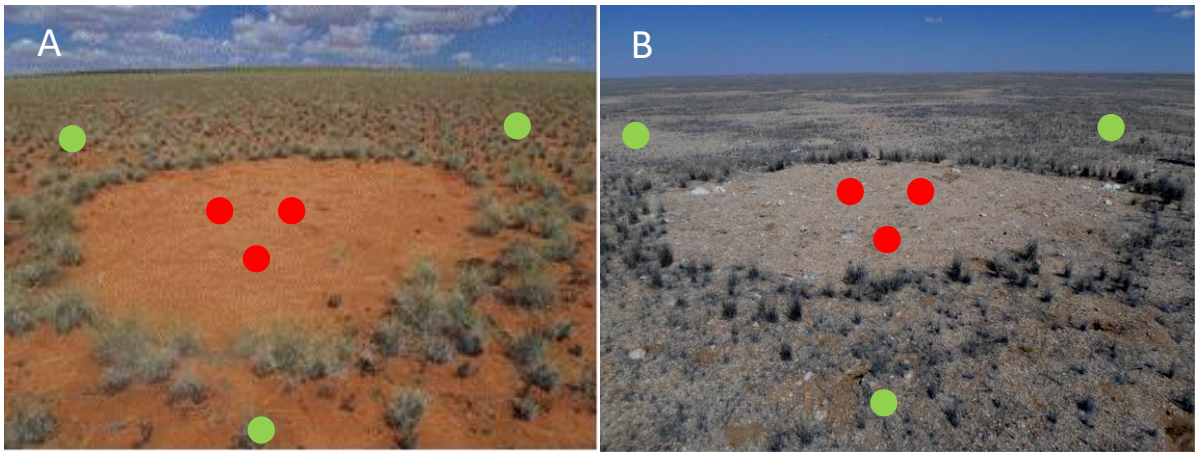

Figure 1. Photographs of Namib Desert dune (A) and gravel plain (B) fairy circles (courtesy of J-B Ramond). Three pseudoreplicates were taken from both inside the fairy circle (red circles) and the control vegetated matrix (green circles).

ammonium (NH_4) and nitrate (NO_3) contents were analysed by Bemlab (Pty) Ltd (Strand, Western Cape, South Africa) using standard protocols. Percentage carbon was determined using the Walkey-Black method (18) and particle size using the hydrometer method (19) at the Department of Plant Production and Soil Science, University of Pretoria.

Metagenomic DNA extraction and MiSeq amplicon sequencing

Metagenomic DNA was extracted from all samples using the PowerSoil® DNA Isolation Kit (MoBio laboratories, CA, USA) according to the manufacturer's instructions. DNA concentrations were determined using a Qubit™ 3.0 Fluorometer (ThermoFisher Scientific, USA). The V4 variable region of the bacterial/archaeal16S rRNA gene was PCR amplified using the 515F (5'-GTG**Y**CAGCMGCCGCGGTAA-3')/806R (5'-GGACTAC**N**VGGGTWTCTAAT-3') primer set (20), and the fungal ITS1 gene region using the ITS1 (5'-TCCGTAGGTGAACCTGCGG-3')/ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') primer set (21). Sequencing was performed by a commercial supplier (MrDNA, Shallowater, TX, USA) on an Illumina MiSeq platform according to manufacturer's guidelines.

MiSeq sequencing analyses

Sequence data analysis was performed using the QIIME (v.1.8.0) platform (22). Low-quality sequences were removed using default parameters, during sequence demultiplexing reads were truncated if individual bases had a Phred score of less than 20

for 6 and 3 consecutive reads for the ITS region and 16S rRNA gene datasets respectively. Chimeric sequences were removed using the usearch61 method. Sequences were clustered into operational taxonomic units (OTUs) using the UCLUST-based-open references OTU clustering pipeline with a 97% sequence identity cut-off (22), against the Greengenes database (23) for 16S rRNA gene and the UNITE database (24) for the ITS region. Bacterial and archaeal taxonomy was assigned to 16S rRNA gene representative sequences using the UCLUST method and the Greengenes reference database. Fungal taxonomy was assigned to ITS sequences using the RDP classifier (25) and the UNITE database.

Statistical analyses

Statistical analyses were performed using the R statistical environment (26) and with PRIMER 6 software (PRIMER-E Ltd, Ivybridge, UK). Soil physicochemistry data were analysed by a Draftmans plot (27) to assess skewness of the data after which K, Cl and % Silt were log(x + 1) transformed. Following data normalization, a Euclidean distance matrix was generated. Principal component analysis (PCA) plots were constructed to assess the relationships among samples, and a permutational analysis of variance (PERMANOVA) test was used to identify significant differences between zones (i.e. dune FC, dune control, gravel plain FC and gravel plain control).

Samples were randomly subsampled to 14,000 and 34,000 reads for the 16S rRNA gene and ITS regions datasets, respectively. Singletons were removed prior to subsequent analyses. Measures of α- (total number of OTUs, Shannon and Simpson indices), β-

(Whittaker) and γ-diversity were performed using the *vegan* package in R (28). Differences in diversity between zones were assessed by analysis of variance (ANOVA) and Tukey HSD post-hoc tests. Biological datasets were square root transformed prior to community structure analysis. Non-metric multidimensional scaling (nMDS) ordination plots were generated from 16S rRNA gene and ITS region datasets using Bray-Curtis dissimilarity matrices, and PERMANOVA was performed to identify significant differences between the community structures of each zone. Abiotic drivers of microbial community structure were assessed by redundancy analysis (RDA) using the *vegan* package in R (26, 28). Plots were constructed using the *ggplot2* package in R (26, 29).

Accession numbers

All sequence data generated as part of this study have been deposited in the Short Read Archive (SRA) of the National Centre for Biotechnology Information under the accession number SRP069846.

Results

Namib Desert Soil Properties

The soil physicochemical properties of the four zones studied (FC and vegetated control soils from both dunes and gravel plains) are summarized in Table 1. A Principal Component Analysis (PCA) separated the samples by site (dune vs. GP) along PC1, explaining 47.4% of the variation observed (separated by a dashed line in Fig. 2). This difference was shown to be statistically significant using PERMANOVA (pseudo-F > 29.25; $P < 0.001$). Overall, soil physicochemical properties were significantly different

Table 1. Soil physicochemical properties of the dune and gravel plain fairy circle centres and their respective vegetated controls.

	Dune		Gravel Plain	
	Control (n = 5)	FC Centre (n = 5)	Control (n = 5)	Centre (n = 5)
pH	7.0 (± 0.2)	7.2 (± 0.2)	6.9 (± 0.1)	7.0 (± 0.2)
Conductivity (mS/m)	8.62 (± 0.49)	9.22 (± 0.91)	8.50 (± 1.04)	9.59 (± 1.71)
P (mg/l)	0.30 (± 0.11)	0.19 (± 0.02)	0.63 (± 0.19)	0.40 (± 0.06)
Na (mg/l)	1.38 (± 0.29)	1.34 (± 0.18)	4.31 (± 0.80)	5.70 (± 1.48)
K (mg/l)	3.71 (± 0.57)	5.63 (± 5.41)	7.25 (± 5.60)	4.91 (± 1.01)
Ca (mg/l)	12.72 (± 0.82)	14.13 (± 1.07)	9.27 (± 1.24)	9.78 (± 1.26)
Mg (mg/l)	1.96 (± 0.28)	2.12 (± 0.13)	2.20 (± 0.47)	2.28 (± 0.16)
Cl (mg/l)	6.16 (± 0.62)	13.32 (± 15.74)	15.97 (± 17.12)	10.24 (± 3.46)
SO_4 (mg/l)	1.86 (± 0.38)	1.57 (± 0.18)	3.96 (± 1.02)	4.09 (± 0.70)
NH_4 (mg/l)	0.32 (± 0.02)	0.35 (± 0.04)	0.37 (± 0.02)	0.36 (± 0.03)
NO_3 (mg/l)	0.36 (± 0.26)	0.21 (± 0.11)	ND	0.52 (± 0.63)
% Carbon	0.03 (± 0.02)	0.03 (± 0.02)	0.08 (± 0.01)	0.05 (± 0.01)
% Clay	ND	ND	3.53 (± 2.24)	3.02 (± 1.73)
% Silt	2.09 (± 1.32)	2.97 (± 1.72)	5.74 (± 1.11)	4.79 (± 1.68)
% Sand	97.91 (± 1.32)	97.03 (± 1.72)	90.73 (± 2.61)	92.19 (± 2.22)

Values are given as mean of ± SD. ND: Not determined, i.e. below the detection limit.

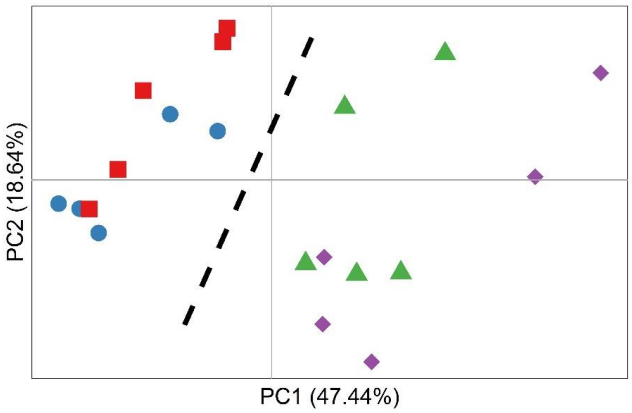

Figure 2. Principal component analysis of physicochemical data from fairy circle and control soils based on a Euclidean distance matrix. Samples are indicated by ■ for dune FC centre, ● for dune control, ▲ for gravel plain FC centre and ♦ for gravel plain control. The dashed line indicates the significant difference observed between gravel plain and dune soil physicochemical properties ($P < 0.001$).

between gravel plain and dune soils (e.g., Na [$P < 0.0001$], Ca [$P < 0.0001$], SO_4 [$P < 0.0001$] and percentage carbon [$P < 0.005$]). Dune soils were of a larger particle size than gravel plain soils (sand: 91.5% ± 2.5% vs 97.5% ± 1.6%; silt: 5.3% ± 1.5% vs 2.5% ± 1.6%; $P < 0.05$ and clay: 3.3% ± 2.0% vs 0.0% [below detection limit]; $P < 0.05$). However, at both sites, the soil physicochemistries of the different zones sampled (FC vs. control soils) could not be statistically discriminated (pseudo-F < 0.31; $P > 0.05$; Fig. 2).

Namib Desert Soil Microbial Community Structures

Illumina MiSeq sequencing generated a total of 537,488 raw reads for the 16S rRNA gene amplicon data set and 1,322,502 raw reads for the ITS region. Sequences were randomly subsampled to 14,000 reads and 34,000 reads per sample for the 16S rRNA gene and for the ITS region datasets, respectively (Fig. A1). After subsampling, an average of 1,081 (± 87) bacterial, 35 (± 4) archaeal and 538 (± 203) fungal OTUs were assigned per sample based on 97% percentage identity cut-off values (Table 2).

Gravel plain bacterial, archaeal and fungal communities were distinct from their dune counterparts on NMDS plots (Fig. 3). PERMANOVA tests confirmed that the communities were significantly different from each other (bacterial community: pseudo-F > 6.06, $P < 0.001$; archaeal community: pseudo-F > 10.37; $P < 0.001$; fungal community: pseudo-F > 6.16; $P < 0.001$). Furthermore, within the sites sampled, we found the edaphic bacterial and archaeal communities from dune FC and control vegetated soils to be significantly different (PERMANOVA, pseudo-F > 2.98; $P < 0.05$ and pseudo-F > 2.59; $P < 0.05$, respectively). In contrast, the gravel plain FC and control communities were not ($P >$

Table 2. α- (Species number, Shannon and Simpson), β- (Beta whittaker) and γ-diversities of bacterial, archaeal and fungal communities in each of the four microenvironments studied.

		Species number	*Shannon*	*Simpson*	*Beta whittaker*	*Gamma diversity*
Bacteria	Dune Control	1153.8 (± 69.8)	5.90 (± 0.07)	0.992 (± 0.001)	1.521	1755
	Dune Centre	1018.6 (± 104.6)	5.79 (± 0.19)	0.988 (± 0.006)	1.683	1714
	GP Control	1046.4 (± 45.4)	5.72 (± 0.05)	0.992 (± 0.001)	1.662	1756
	GP Centre	1103.2 (± 35.3)	5.84 (± 0.14)	0.991 (± 0.005)	1.678	1834
Archaea	Dune Control	34.2 (± 2.7)	2.07(± 0.26)	0.767 (± 0.066)	1.433	49
	Dune Centre	35.6 (± 4.5)	2.14 (± 0.22)	0.777 (± 0.079)	1.404	50
	GP Control	32.8 (± 2.2)	2.39 (± 0.11)	0.865 (± 0.017)	1.524	50
	GP Centre	36.4 (± 3.4)	2.24 (± 0.30)	0.825 (± 0.065)	1.484	54
Fungi	Dune Control	553.0 (± 88.0)	2.88 (± 0.46)	0.842 (± 0.077)	1.951	1079
	Dune Centre	366.4 (± 59.0)	2.61 (± 0.42)	0.832 (± 0.070)	4.539	1663
	GP Control	586.6 (± 80.6)	3.00 (± 0.11)	0.870 (± 0.0152)	3.074	1803
	GP Centre	613.0 (± 100.8)	3.20 (± 0.32)	0.888 (± 0.042)	2.845	1744

Values given as mean of ± SD of 5 measurements

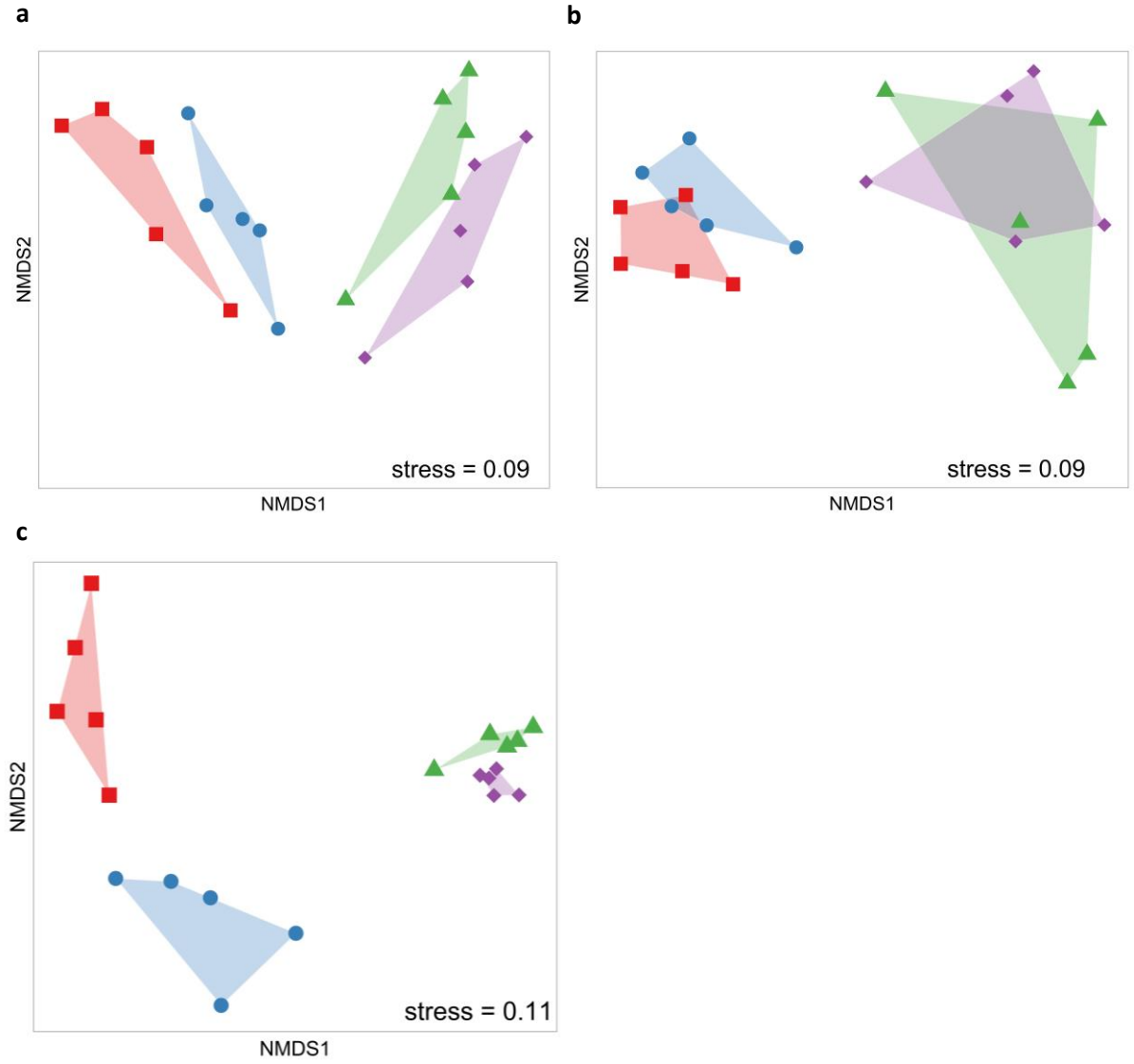

Figure 3. Two dimensional non-metric multidimensional scaling (NMDS) plot of bacterial (a), archaeal (b) and fungal (c) community structures based on Bray Curtis similarity matrices of square root transformed 16S rRNA gene (bacteria and archaea) and ITS region (fungi) OTU relative abundance tables. Dune FC centre communities are indicated by ■, dune control by ●, gravel plain FC centre by ▲ and gravel plain control by ♦. Samples from similar zones are shaded according to colour to indicate clustering.

0.05). The fungal communities were all found to be zone-specific, i.e., were significantly different between the dune FC, dune control, gravel plain FC and gravel plain control soils (pseudo-F > 2.33; P <0.05; Fig. 3).

Environmental Drivers of Soil Community Composition

Redundancy analysis (RDA) biplots were used to assess physicochemical factors that influenced the microbial community structures (Fig. 4). Overall, the combination of variables explained 61.7%, 80.6% and 55.6% of the bacterial (Fig. 4a), archaeal (Fig. 4b) and fungal (Fig. 4c) community structure variances, respectively. All communities were clearly separated along the first axis according to their sampling site and were significantly influenced by the same nine physicochemical variables (with the exception of archaea which were also influenced by two additional properties [Mg and NH_4; both $P < 0.05$]). Percentage of sand, pH and Ca content ($P < 0.001$, $P < 0.05$ and $P < 0.001$, respectively) were found to be determining factors in shaping the dune microbial communities, whereas the gravel plain communities were significantly influenced by P, Na and S concentrations as well as carbon, silt and clay percentages ($P < 0.005$, $P < 0.001$, $P < 0.001$, $P < 0.05$, P <0.005 and $P < 0.05$, respectively). However, we found no soil parameters that significantly influenced FC and control microbial communities (Fig. 4), suggesting that other unmeasured factors, either biotic or abiotic, may be involved.

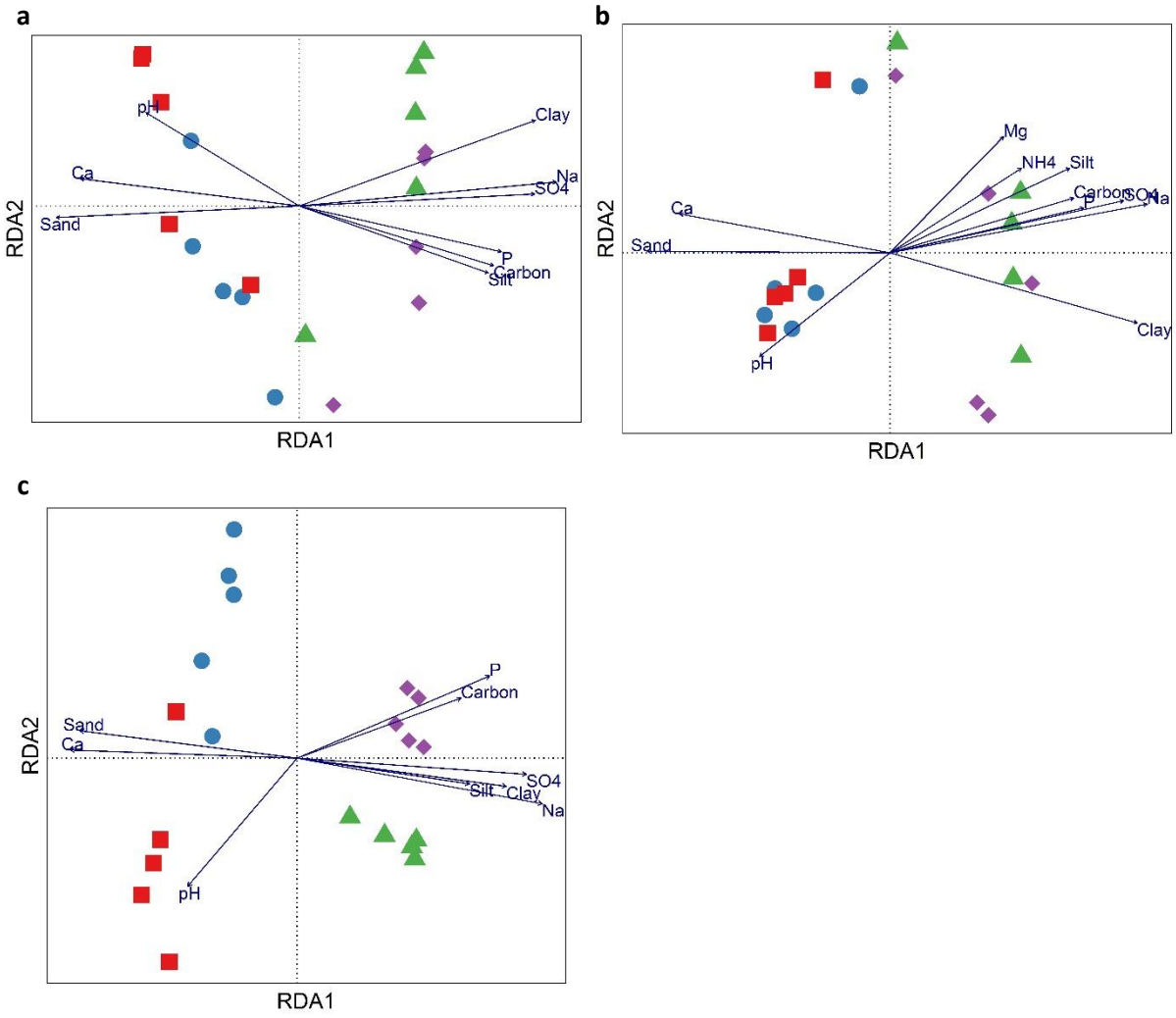

Figure 4. Redundancy analysis (RDA) plots showing the influence of soil physicochemical properties on (a) bacterial, (b) archaeal and (c) fungal communities. Vectors indicate significant ($p < 0.05$) correlations between soil physicochemical properties and microbial community composition. Dune FC centre communities are indicated by ■, dune control by ●, gravel plain FC centre by ▲ and gravel plain control by ♦.

Bacterial, Archaeal and Fungal Richness and Diversity

Analysis of the 16S rRNA gene and ITS region amplicon datasets showed that Namib Desert edaphic bacterial communities were more diverse than fungal and archaeal communities (Table 2), having significantly higher species number (ANOVA, $F > 654.5$; $P < 0.001$), Shannon ($F > 788.4$; $P < 0.0001$) and Simpson ($F > 56.1$; $P < 0.001$) indices.

Bacterial and archaeal communities showed consistent levels of species richness across the two zones of the two sites studied (i.e., gravel plain and dune FC and control soils; ANOVA, $P > 0.05$), whereas fungal communities had significantly higher species numbers in the gravel plains than in the dunes (ANOVA, $F > 7.74$; $P < 0.05$). Both bacterial and archaeal communities also presented high levels of evenness in all of the zones sampled, with no significant differences in α-diversities between zones sampled (ANOVA, $P > 0.05$). Phylogenetic heterogeneity within zones (β-diversity) ranged from 1.52 to 1.69 (β-Whittaker index) for bacterial communities, and from 1.40 to 1.53 for archaeal communities. A large number of bacterial and archaeal OTUs were also shared between all four zones (59.2% and 58.3%, respectively; Table 2, Figure 5a and 5b).

In contrast, fungal communities were highly variable in diversity with significantly different species richness estimates across the four different zones studied (ANOVA, $F > 7.15$; $P < 0.005$). The dune FC soils (366 ± 59 OTUs) had a significantly lower number of fungal OTUs than all the other sampled zones (dune control [553 ± 88 OTUs, Tukey post-hoc test; $P < 0.05$], gravel plain FCs [613 ± 101 OTUs; $P < 0.01$] and gravel plain control [587 ± 81 OTUs, $P < 0.005$]). Fungal communities had large numbers of zone-specific OTUs (Table 2; Figure 5c), suggesting that differential assembly processes occur for fungal communities in each of the Namib Desert soil environments studied. This is further

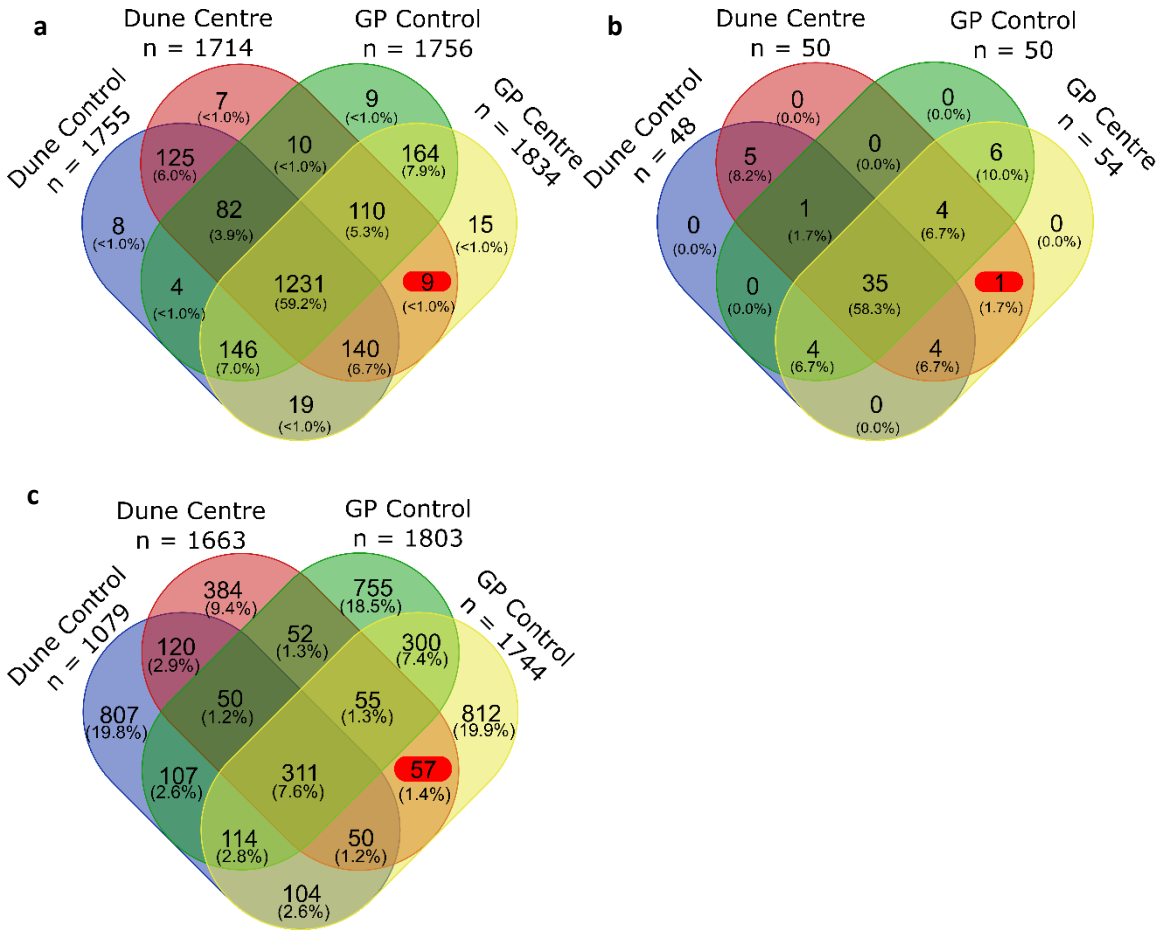

Figure 5. Venn diagrams showing the distribution of bacterial (a), archaeal (b) and fungal (c) OTUs within the studied soils. Overlap indicates co-occurrence of an OTU in more than one microenvironment. OTUs solely present in both the dune and gravel plain FC centres are indicated in red.

emphasized by the fact that β-Whittaker indices, which ranged from 1.95 to 4.54, were significantly different in each zone studied (ANOVA, $F > 17.14$; $P < 0.001$).

Globally, Actinobacteria (25-38%), Proteobacteria (16-33%) and Crenarchaeota (10-25%) were the most abundant bacterial and archaeal phyla in all zones (Fig A2a) while moderately abundant taxa included Acidobacteria (2-5%), Bacteroidetes (3-20%), Chloroflexi (2-4%), Gemmatimonadetes (1-2.5%), Verrucomicrobia (0.9-2.8%) and Firmicutes (<0.1-2.8%). As expected, and based on the diversity indices (Table 2), the compositions of fungal communities were highly variable between samples, as each of the four zones studied were dominated by a unique fungal class (gravel plain control: fungi of class Dothideomycetes [46.2%]; gravel plain centre: Dothideomycetes [27.5%]; Dune control: fungi of class Agaricostilbomycetes [23.2%]; Dune centre: fungi of phylum Chytridiomycota [43.6%]; Fig. A2b). Globally, fungi of the phylum Chytridiomycota were significantly more abundant in dune soils compared to gravel plain soils (Student's T-test, $P < 0.05$), while Dothideomycetes displayed an opposite trend ($P < 0.01$).

Fairy Circle-Specific Phylotypes

We identified 9 bacterial, 1 archaeal and 57 fungal phylotypes which were FC-specific; i.e., their phylotypic signals were found only within soils of the dune and the gravel plain FCs (see Fig. 5). Their phylogenetic assignments are given in Table 3. The FC-specific archaeal OTU was related to the ammonia-oxidizing *Nitrososphaera* genus from the Crenarchaeota phylum. The bacterial OTUs were assigned as Cyanobacteria (4/9), Firmicutes (2/9), Proteobacteria (2/9) and Actinobacteria (1/9). Although 33 of the 57

Table 3. Identified FC-specific OTUs, i.e., observed in both the dune and gravel plain fairy circles. OTUs are classified to the lowest level possible using a high (>80%) level of sequence similarity.

Kingdom	Phylum	Class	Order	Family	Genus	Species
Archaea	Thaumarchaeota	Nitrosophaeria	Nitrososphaerales	Nitrososphaeraceae	*Candidatus,Nitrososphaera*	
Bacteria	Actinobacteria	Nitriliruptoria	Nitriliruptorales	Nitriliruptoraceae		
	Cyanobacteria	Oscillatoriophycideae	Chroococcales	Xenococcaceae		
		Chloroplast	Stramenopiles			
		Unclassified				
		Unclassified				
	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	
				Paenibacillaceae	*Brevibacillus*	
	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae		
		Gammaproteobacteria				
Fungi	Ascomycota	Dothideomycetes	Pleosporales	Incertae sedis	*Periconia*	
				Incertae sedis	*Phoma*	
				Montagnulaceae	Unidentified	
				Pleosporaceae	*Curvularia*	
					Curvularia	
					Curvularia	
				Sporormiaceae	Unclassified	
		Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*Aspergillus flavus*
			Onygenales	Unidentified		
		Incertae sedis	Incertae sedis	Incertae sedis	*Calcarisporiella*	
		Unclassified				
		Sordariomycetes	Hypocreales	Incertae_sedis_3	*Stachybotrys*	*Stachybotrys microspora*
			Sordariales	Chaetomiaceae	Unclassified	
			Xylariales	Unclassified		
			Xylariales	Unclassified		
	Basidiomycota	Agaricomycetes	Agaricales	Entolomataceae	*Clitopilus*	
	Chytridiomycota	Chytridiomycetes	Unclassified			
			Rhizophlyctidales	Rhizophlyctidaceae	*Rhizophlyctis*	
					Rhizophlyctis	*Rhizophlyctis rosea*
					Rhizophlyctis	*Rhizophlyctis rosea*
					Rhizophlyctis	*Rhizophlyctis rosea*
					Rhizophlyctis	*Rhizophlyctis rosea*
		Unclassified				
		Unclassified				

(~58%) FC-specific fungal OTUs could not be classified below the kingdom level, 15 were classified as Ascomycota, 1 as Basidiomycota and 8 as Chytridiomycota. 5 of the 8 Chytridiomycota were of the *Rhizophlyctis* genus and 7 of the Ascomycota belonged to the Dothideomycetes class. Some of the Ascomycota-assigned OTUs belonged to the *Periconia*, *Phoma* and *Curvularia* genera and a single OTU was assigned as *Aspergillus flavus* (Table 3).

Discussion

Microorganisms play important roles in desert environment bioprocesses, including biogeochemical cycling of carbon and nitrogen and bioweathering of exposed bedrock (30, 31). In addition, they can interact positively (e.g., as mycorrhizae (32)) or negatively (e.g., as phytopathogens (33)) with plants. We thus hypothesize that some microbial groups found in FCs may interact negatively with plant species through phytopathogenic effects, and thereby play a role in the formation and/or maintenance of these enigmatic features found in the Namib Desert. A corollary to this hypothesis is that FC-specific taxa should be present in the soils of both the gravel plain and dune FCs, and absent from the surrounding vegetated soils (2, 16).

In agreement with previous desert phylogenetic surveys, we identified Actinobacteria as the most abundant phylum in our soil samples (30). We also found relatively high abundances of Proteobacteria, Acidobacteria, Bacteroidetes and Chloroflexi in all the Namib Desert soil zones studied, which is broadly consistent with other Namib Desert surveys (30, 34, 35). We report a consistently high relative abundance of a limited number

of archaeal phylotypes, as observed in Sonoran Desert soils (36). However, a high abundance of Thaumarchaeota (which was recently reclassified from Crenarchaeota (37)) was detected in Namib Desert soils, while the crenarchaean Thermoproteus taxa dominated the Sonoran desert samples (35).

Fungal diversity has not been comprehensively surveyed in desert environments worldwide (30). In the Namib Desert, mycorrhizal- (38) and lichen-associated (39) fungi have been shown to have the potential to participate in local biogeochemical cycling, specifically by decomposing surface litter (40). Although we were only able to classify approximately 50% of the fungal OTUs (due to the under-representation of environmental sequences in the UNITE database; (41)), this is the first study within the Namib Desert to use next generation high throughput sequencing to assess fungal communities. We observed that Ascomycota, Basidiomycota and Chytridiomycota were ubiquitously detected in these desert soils. Dominant fungal genera, identified as *Aspergillus, Chaetomium, Pleosporales* and *Stachybotrys*, are well-known desert colonists (42).

Microbial Community Assembly in Namib Desert Soils

In hyperoligotrophic desert environments, soil physicochemical properties including water content (43), soil pH (44), carbon content (45), nitrate concentration (46), micronutrients (47) and particle size (44) have been shown to significantly shape microbial community structures. In a comparative T-RFLP fingerprinting analysis (48), Namib Desert dune and gravel plain bacterial communities were shown to be significantly different from one another, and microbial community structures were significantly governed by soil pH, Na

content and % silt and sand. Using high-throughput sequencing, we demonstrate that bacterial, archaeal and fungal communities of Namib Desert dune fields and gravel plains are significantly different. We found that percentage sand, pH and Ca significantly shaped dune microbial (bacterial, archaeal and fungal) communities, whereas soil P, Na, S content, percentage clay, silt and carbon shaped the gravel plain communities. Overall, this supports the concept that deterministic processes play important roles in shaping Namib Desert soil microbial communities, with microbial communities at each site adapting to local physicochemical conditions (49).

None of the soil physicochemical properties measured were significantly different between FC and control soils in both the dunes and gravel plain, which is expected given the close proximity of each FC and control sample. Consequently, the measured soil properties could not explain the differences observed between FC and vegetated control soil microbial communities. This suggests the potential involvement of other factors, such as other edaphic properties (e.g., Fe, Zn and/or Al contents (48)), the presence of a toxin (50), or unknown stochastic processes (51).

Namib Desert dune and gravel plain bacterial and archaeal communities shared a large numbers of OTUs between the four zones sampled (59.2% and 58.3%, respectively), which is much higher than previously observed for Namib Desert microbial communities (35). This large set of cosmopolitan edaphic bacterial and archaeal phylotypes is indicative of a Namib Desert core community, not subject to dispersal limitation. This indicates that these microbial communities may also be influenced by stochastic (random) events, such as the transport of viable cells over large distances by wind dispersal, rather than deterministic processes, such as differences in soil physicochemical properties.

In contrast, fungal communities were ‘zone-specific’, with each zone containing a high number of unique OTUs. Fungal communities also showed a clear and significant distinction between the dune and gravel plain sites. In addition, FC and control vegetated soil fungal communities were significantly different in fungal composition, a pattern that was not as clearly observed with the distribution of bacterial and archaeal in the zones sampled. Because there is no significant difference between soil physicochemical properties of FC and control soils, we conclude that FC-specific fungal communities are selected through habitat-filtration (vegetated soil vs barren soil) (16) or an environmental disturbance (such as the release of a toxin (50)). It is known that the assembly of edaphic bacterial and archaeal communities may differ from that of fungal communities (52, 53).

Could edaphic microbial communities be involved in the Fairy Circle phenomenon?

While many hypotheses have been proposed in attempts to explain the origin of Namib Desert Fairy Circles (2, 4, 8, 10, 11, 13-15), none take account of the presence and differences of Fairy Circles in the gravel plains and dune environments. Considering that almost all FC studies have focussed exclusively on dune FCs, we compared microbial communities in both dune and gravel plain FCs.

Our hypothesis that microorganisms may play a role in FC etiology is based on the fact that both bacteria and fungi can have pathogenic or phytotoxic capacities (33, 54). Furthermore, microorganisms have been implicated in the formation of various FC-like phenomenon, such as the dollar spot (17), fairy rings (55) and circular patches in intertidal

mats (56). Additionally, plant pathogens have been shown to be viable and able to infect plants in desert environments (e.g. the fungi *Alternaria solani, Stemphylium* spp. and *Botrytis cinerea* as well as a novel virus from the *Potyvirus* genus in *Albuca rautanenii* found in the Namib Desert) (33, 57).

We identified 10 and 57 OTUs from the 16S rRNA gene and ITS region datasets, respectively, to be exclusively and consistently present within dune and gravel FC soils. Of these FC-specific OTUs, many were assigned to phylogenetic groups harbouring known phytotoxic or phytopathogenic microorganisms, such as the fungal *Periconia, Culvularia* and *Aspergillus* genera, the fungal Pleosporales order, the fungal Chaetomiaceae family and the bacterial Gammaproteobacteria class (58-63). While these results do not directly implicate microorganisms in the creation and/or maintenance of Namib Desert FCs, they remain consistent with the hypothesis that microorganisms play a role in the phenomenon.

Conclusions

Here, we report the first phylogenetic study to compare Fairy Circles from both the Namib Desert gravel plains and dune fields. Overall, the Namib Desert edaphic bacterial communities were more diverse than the archaeal and fungal communities. Bacterial and archaeal communities showed consistent microbial community diversity across both soil types. The high proportions of shared community members between soil types suggests that stochastic processes are important in their assembly (51). In contrast, fungal communities were more variable overall, with unique sets of fungal phylotypes detected

in each zone sampled. This may suggest that deterministic niche adaptation is the primary mechanism in their assembly (49). Finally, we have identified microorganisms solely present in FC soils, which may play a role in the Fairy Circle phenomenon. Many of the OTUs found only in FCs are related to known phytopathogen groups (fungal genera *Periconia, Culvularia and Aspergillus*, order Pleosporales and family Chaetomiaceae, as well as bacterial Gammaproteobacteria class (58-63)), suggesting that they may play a role in this phenomenon. To further investigate this hypothesis, the FC-specific phylotypes should be isolated and used in controlled greenhouse experiments to identify patterns related to phytopathology (64). The use of function-based approaches, such as metaproteomics and/or metagenomics (65, 66), could also assist in the identification of phytotoxic proteins or toxins, as well as the enzymes or pathways involved in their biosynthesis.

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Appendixes

Supplementary figure A1.

Supplementary figure A2.

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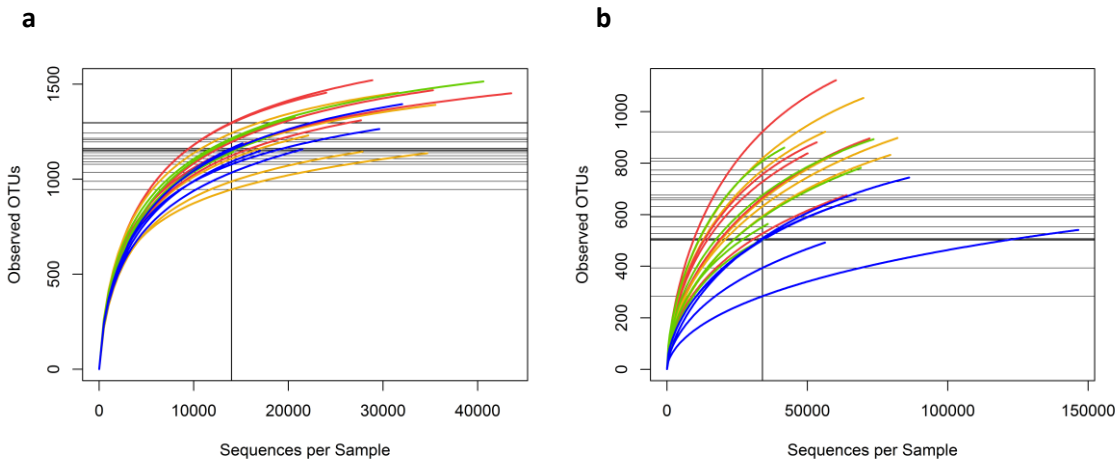
Appendixes

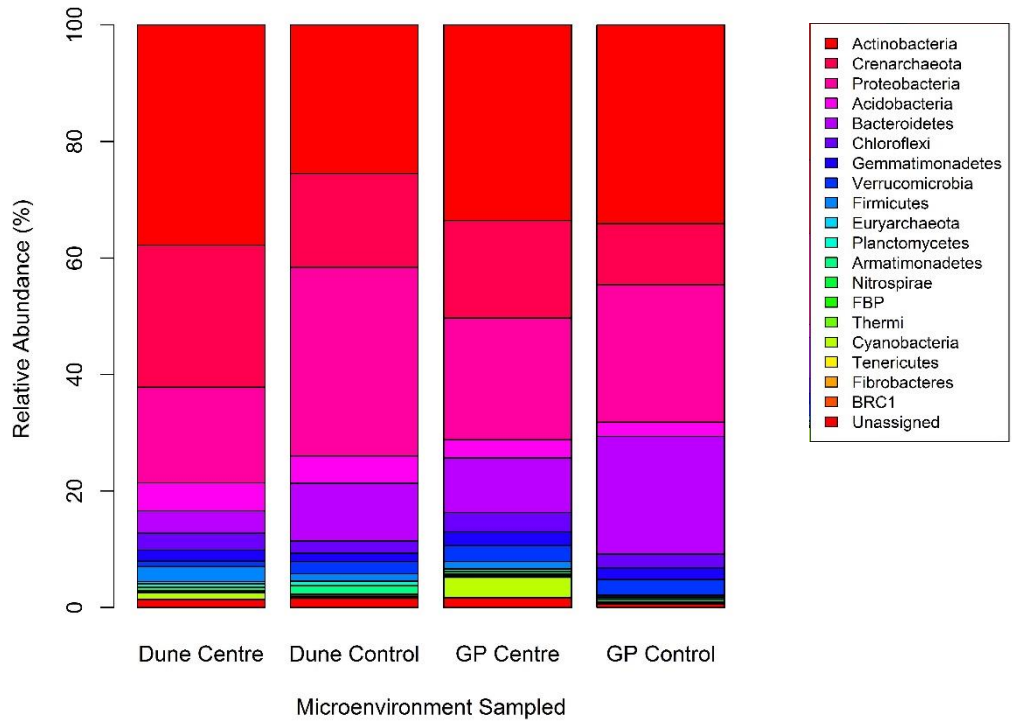
Supplementary figure S1.



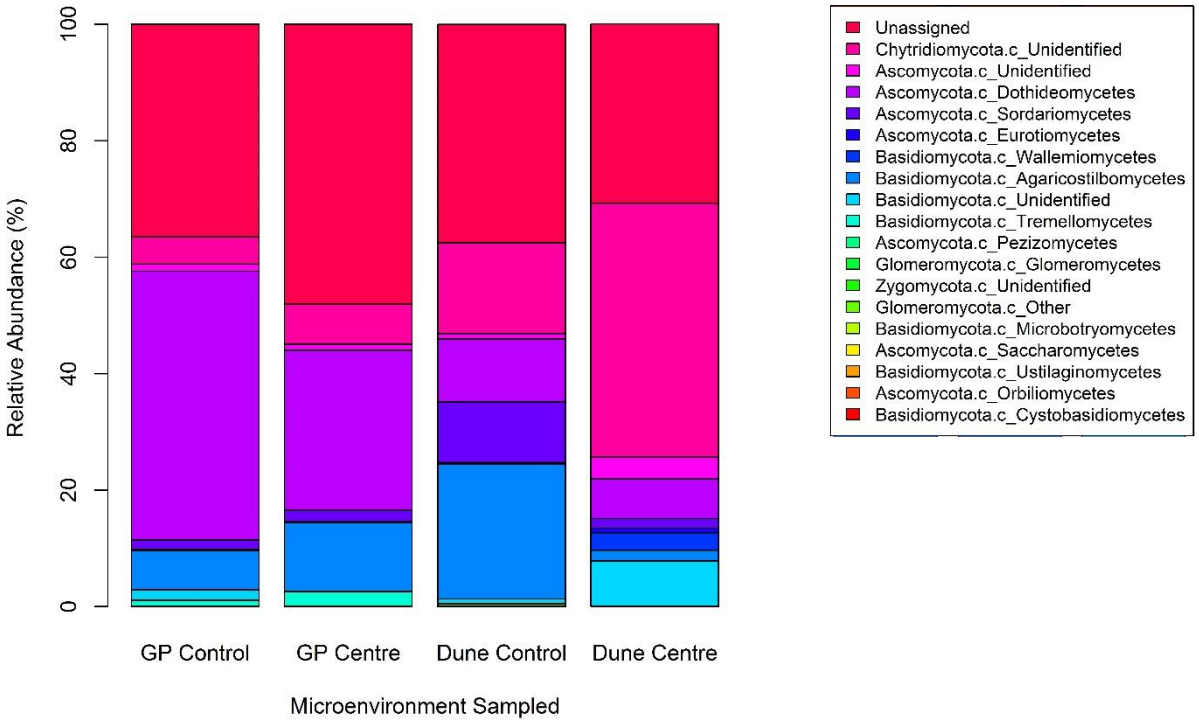
Supplementary figure A1. Rarefaction curves for both the 16S rRNA gene (a) and ITS region (b) datasets. Dune centre samples are indicated in yellow, dune control samples in red, gravel plain centre in blue and gravel plain control in green. Vertical lines indicate the samples with the fewest number of reads, at which normalization took place, and horizontal lines indicate the amount of reads per sample at normalization level.

Supplementary figure S2.

a



b



Supplementary figure A2. Average bacterial and archaeal (a) as well as fungal (b) community taxonomic composition of the studied soils. 16S rRNA gene sequences were classified into 19 bacterial and archaeal phyla with <1,5% of unclassified sequences. ITS sequences were classified into 5 phyla with a large number of reads remaining unassigned to taxonomic levels below kingdom (38.2% ± 17.4%).