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Unique Microbial Phylotypes in Namib Desert Dune and Gravel Plain Fairy Circle Soils

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ABSTRACT

Fairy circles (FCs) are barren circular patches of soil surrounded by grass species. Their origin is poorly understood. FCs feature in both the gravel plains and the dune fields of the Namib Desert. While a substantial number of hypotheses to explain the origin and/or maintenance of fairy circles have been presented, none are completely consistent with either their properties or their distribution. In this study, we investigated the hypothesis that FC formation in dunes and gravel plains is due to microbial phytopathogenesis. Surface soils from five gravel plain and five dune FCs, together with control soil samples, were analyzed using high-throughput sequencing of bacterial/archaeal (16S rRNA gene) and fungal (internal transcribed spacer [ITS] region) phylogenetic markers. Our analyses showed that gravel plain and dune FC microbial communities are phylogenetically distinct and that FC communities differ from those of adjacent vegetated soils. Furthermore, various soil physicochemical properties, particularly the pH, the Ca, P, Na, and SO_4 contents, the soil particle size, and the percentage of carbon, significantly influenced the compositions of dune and gravel plain FC microbial communities, 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, since they were present within the gravel plain and dune FC soils only, not in the vegetated soils. Some of these FC-specific phylotypes were assigned to taxa known to harbor 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 gravel plains of the Namib Desert. Various hypotheses attempting to explain this phenomenon have been proposed. To date, however, none have been successful in fully explaining the etiology of FCs, particularly since 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 the microbial communities in FC and control vegetated soil samples were significantly different. Furthermore, we detected 67 FC-specific microbial phylotypes, i.e., phylotypes present solely in both gravel plain and 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.

Fairy circles (FCs) are circular patches of soil completely devoid of vegetation within a matrix of grass, typically *Stipagrostis* spp. (1, 2). Common in the Namib Desert, and recently observed in the Australian outback (3), FCs are limited to arid areas that receive between 50 and 100 mm precipitation *per annum*. FCs are nonpermanent; they typically appear, enlarge, and disappear, with an estimated life span ranging from 22 to 60 years (4, 5).

Although FCs were first reported in the 1970s (6), there is currently no scientific consensus on the mechanisms behind their formation and maintenance (2, 7–9). Fauna-based hypotheses suggest that FCs result from the harvesting activities of termites, ants, or rodents (1, 5, 10–12). The hypothesis of spatial self-organization of plants 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 focused solely on FCs located in Namib Desert dunes (2, 4, 8, 10, 14, 15), whereas FCs also occur, albeit more sparsely, in 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, because two fungal terminal restriction fragments

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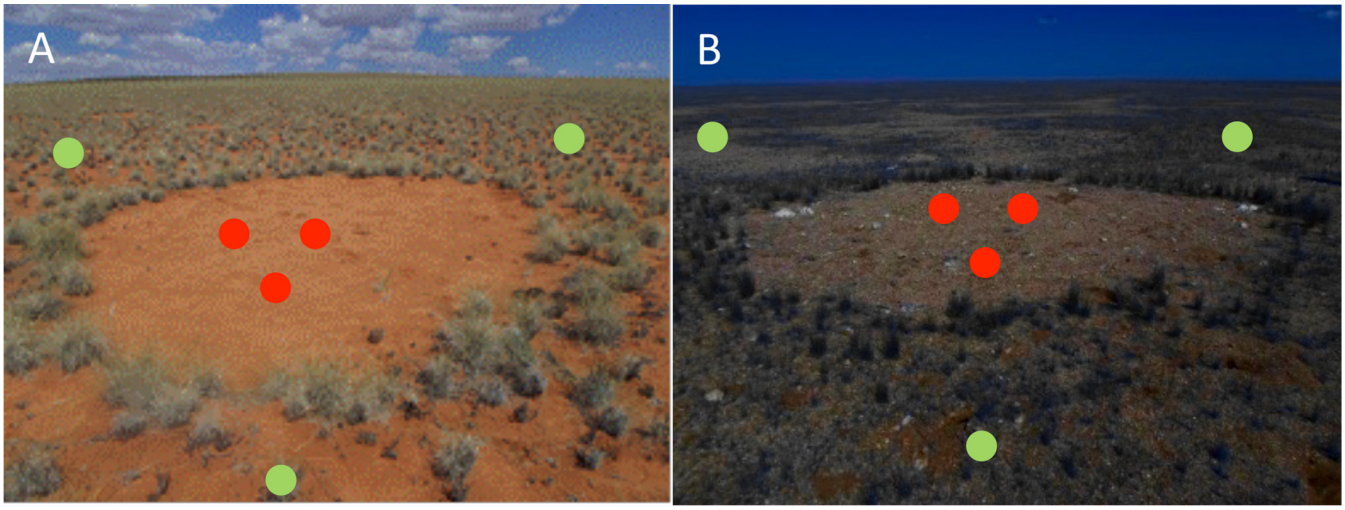

FIG 1 Photographs of Namib Desert dune (A) and gravel plain (B) fairy circles (from J.-B. Ramond). Three pseudoreplicates were taken from inside the fairy circle (red circles) and three from the control vegetated matrix (green circles).

(T-RFs) were detected within gravel plain FCs. However, due to the nature of the fingerprinting technique used, these fragments could not be assigned taxonomic identities (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 that inhibits grass/plant growth (17). Although contested by the findings of recent *in situ* soil transfer experiments for five dune FCs (7), the phytotoxicity of FC soils has been shown in greenhouse experiments (2), an observation 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 dune FCs with gravel plain FCs. We used Illumina MiSeq high-throughput sequencing of the 16S rRNA gene and the internal transcribed spacer (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 present solely within dune and gravel plain FC 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 (~400 g) of surface soils (depth, 0 to 5 cm) were collected aseptically from the center of each of five individual FCs and from adjacent vegetated matrix sites (at a distance of twice the radius from the FC center) ($n = 60$) (Fig. 1). For each set of soil samples, the three pseudoreplicates were combined in equal amounts (~100 g), for a total of of 20 samples. The samples were transported and were 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 were sieved (2 mm) prior to analysis. The soil pH, conductivity, and sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), chloride (Cl), sulfate (SO_4), phosphorus (P), ammonium (NH_4), and nitrate (NO_3) contents were analyzed by Bemlab (Pty) Ltd. (Strand, Western Cape, South Africa) using standard protocols. The percentage of carbon was determined by use of the Walkley-Black method (18), and the particle size by use of 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 (Mo Bio Laboratories, CA, USA) according to the manufacturer's instructions. DNA concentrations were determined using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA). The V4 variable region of the bacterial/archaeal 16S rRNA gene was amplified by PCR using the 515F (5′-GTG**Y**CAGCMGCCGCGGTAA-3′)/806R (5′-GGAC TAC**N**VGGGTWTCTAAT-3′) primer set (where **N** = A, T, C, or G and **Y** = C or T) (20), and the fungal ITS1 gene region was amplified by PCR using the ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′)/ITS2 (5′-GCTGC GTTCTTCATCGATGC-3′) primer set (21). Sequencing was performed by a commercial service provider (MR DNA, Shallowater, TX, USA) on an Illumina MiSeq platform according to the manufacturer's guidelines.

MiSeq sequencing analyses. Sequence data analysis was performed using the QIIME (version 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 <20 for 6 consecutive reads for the ITS region data set or for 3 consecutive reads for the 16S rRNA gene data set. Chimeric sequences were removed using the usearch61 method. Sequences were clustered into operational taxonomic units (OTUs) using the UCLUST-based open-reference OTU clustering pipeline with a 97% sequence identity cutoff (22) against the Greengenes database (23) for the 16S rRNA gene and against the UNITE database (24) for the ITS region. Bacterial or archaeal taxonomy was assigned to representative 16S rRNA gene 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) with PRIMER software (version 6; Primer-E Ltd., Ivybridge, United Kingdom). Soil physicochemical data were analyzed by a draftsman plot (27) to assess the skewness of the data, after which the K and Cl contents and the percentage of 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

TABLE 1 Physicochemical properties of the soils of the dune and gravel plain fairy circle centers and their respective vegetated controls

Property	Value for soil[a]			
	Dune		Gravel plain	
	Control	FC center	Control	FC center
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)
Concn (mg/liter) of:				
P	0.30 (± 0.11)	0.19 (±0.02)	0.63 (±0.19)	0.40 (±0.06)
Na	1.38 (±0.29)	1.34 (±0.18)	4.31 (±0.80)	5.70 (±1.48)
K	3.71 (±0.57)	5.63 (±5.41)	7.25 (±5.60)	4.91 (±1.01)
Ca	12.72 (±0.82)	14.13 (±1.07)	9.27 (±1.24)	9.78 (±1.26)
Mg	1.96 (±0.28)	2.12 (±0.13)	2.20 (±0.47)	2.28 (±0.16)
Cl	6.16 (±0.62)	13.32 (±15.74)	15.97 (±17.12)	10.24 (±3.46)
SO_4	1.86 (±0.38)	1.57 (±0.18)	3.96 (±1.02)	4.09 (±0.70)
NH_4	0.32 (±0.02)	0.35 (±0.04)	0.37 (±0.02)	0.36 (±0.03)
NO_3	0.36 (±0.26)	0.21 (±0.11)	ND	0.52 (±0.63)
% of:				
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)

[a] There were five samples for each type of soil. Values are means (± standard deviations). ND, not determined (i.e., below the detection limit).

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 region data sets, respectively. Singletons were removed prior to subsequent analyses. Measurements of α-diversity (total number of OTUs; Shannon and Simpson indices), β-diversity (Whittaker index), 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's honestly significant difference (HSD) *post hoc* test. Biological data sets were square root transformed prior to community structure analysis. Nonmetric multidimensional scaling (NMDS) ordination plots were generated from 16S rRNA gene and ITS region data sets 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 number(s). All sequence data generated as part of this study have been deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information under accession number SRP069846.

RESULTS

Properties of Namib Desert soils. The physicochemical properties of the soils 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 versus gravel plain) along PC1, explaining 47.4% of the variation observed (Fig. 2, dashed line). This difference was shown to be statistically significant using PERMANOVA (pseudo-$F > 29.25$; $P < 0.001$). Overall, the physicochemical properties differed significantly between gravel plain and dune soils (e.g., in Na [$P < 0.0001$], Ca [$P < 0.0001$], and SO_4 [$P < 0.0001$] contents and in the percentage of carbon [$P < 0.005$]). Dune soils had a larger particle size than gravel plain soils (sand, 91.5% ± 2.5% versus 97.5% ± 1.6%; silt, 5.3% ± 1.5% versus 2.5% ± 1.6% [$P < 0.05$]; clay, 3.3% ± 2.0% versus 0.0% [below the detection limit] [$P < 0.05$]). However, at both sites, the soil physicochemistries of the different zones sampled (FC versus control soils) could not be statistically distinguished (pseudo-$F < 0.31$; $P > 0.05$) (Fig. 2).

Structures of microbial communities in Namib Desert soils. 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 and 34,000 reads per sample for the 16S rRNA gene and ITS region data sets, respectively (see Fig. SA1 in the supplemental material). After subsampling, averages of 1,081 (±87) bacterial, 35 (±4) archaeal, and 538 (±203) fungal OTUs were assigned per sample based on 97% identity cutoff 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 (for the bacterial communities, pseudo-F was >6.06 and P was <0.001; for the archaeal communities, pseudo-F was >10.37 and P was <0.001; for the fungal communities, pseudo-F was >6.16 and P was <0.001). Furthermore, within the sites sampled, we found that the edaphic bacterial and archaeal communities in dune FC soil differed significantly from those in control vegetated soil by PERMANOVA (for bacterial communities, pseudo-F was >2.98 and P was <0.05; for archaeal communities, pseudo-F was >2.59 and P was <0.05). In contrast, the gravel plain FC and control communities were not significantly different ($P > 0.05$). The fungal communities were all found to be zone specific, i.e., they differed significantly 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 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

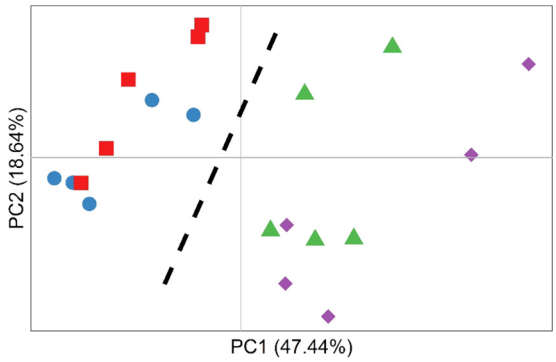

FIG 2 Principal-component analysis of physicochemical data from fairy circle and control soils based on a Euclidean distance matrix. Samples are indicated by colored symbols as follows: red squares, dune FC center; blue circles, dune control; green triangles, gravel plain FC center; purple diamonds, gravel plain control. The dashed line indicates the significant difference observed between the physicochemical properties of gravel plain and dune soils ($P < 0.001$).

TABLE 2 α-, β-, and γ-diversities of bacterial, archaeal, and fungal communities in each of the four microenvironments studied

Community	Microenvironment[a]	α-Diversity[b]			β-Diversity (Whittaker index)	γ-Diversity
		No. of species	Shannon index	Simpson index		
Bacteria	Dune control	1,153.8 (±69.8)	5.90 (±0.07)	0.992 (±0.001)	1.521	1,755
	Dune FC center	1,018.6 (±104.6)	5.79 (±0.19)	0.988 (±0.006)	1.683	1,714
	GP control	1,046.4 (±45.4)	5.72 (±0.05)	0.992 (±0.001)	1.662	1,756
	GP FC center	1,103.2 (±35.3)	5.84 (±0.14)	0.991 (±0.005)	1.678	1,834
Archaea	Dune control	34.2 (±2.7)	2.07 (±0.26)	0.767 (±0.066)	1.433	49
	Dune FC center	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 FC center	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	1,079
	Dune FC center	366.4 (±59.0)	2.61 (±0.42)	0.832 (±0.070)	4.539	1,663
	GP control	586.6 (±80.6)	3.00 (±0.11)	0.870 (±0.0152)	3.074	1,803
	GP FC center	613.0 (±100.8)	3.20 (±0.32)	0.888 (±0.042)	2.845	1,744

[a] GP, gravel plain.
[b] Values are means (± standard deviations) of 5 measurements.

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 contents; P, < 0.05 for both]). The percentage of sand, the pH, and the Ca content (P, <0.001, <0.05, and <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

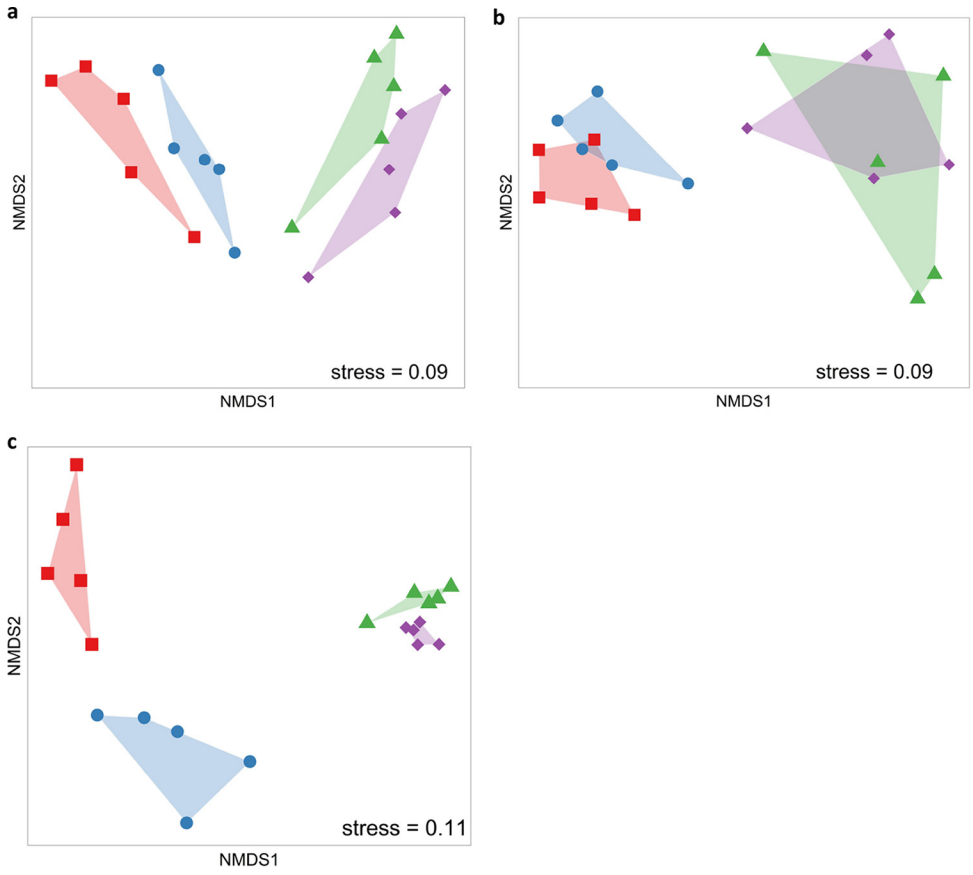

FIG 3 Two-dimensional nonmetric multidimensional scaling (NMDS) plots of bacterial (a), archaeal (b), and fungal (c) community structures based on Bray-Curtis similarity matrices of square root-transformed relative abundances of 16S rRNA gene (bacteria and archaea) and ITS region (fungi) OTUs. Communities are indicated by colored symbols as follows: red squares, dune FC center; blue circles, dune control; green triangles, gravel plain FC center; purple diamonds, gravel plain control. Each area bounded by samples from the same zone is shaded in the corresponding color to indicate clustering.

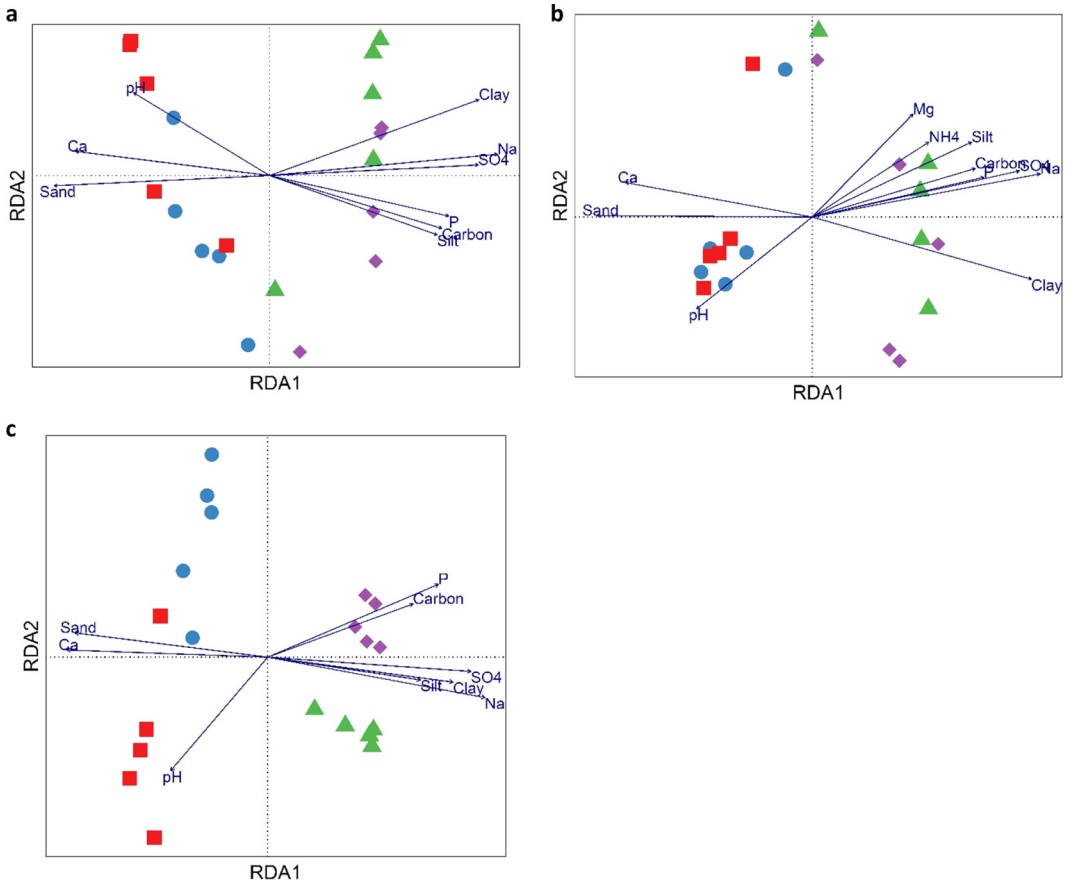

FIG 4 Redundancy analysis (RDA) plots showing the influence of soil physicochemical properties on bacterial (a), archaeal (b), and fungal (c) communities. Vectors indicate significant ($P < 0.05$) correlations between soil physicochemical properties and microbial community composition. Communities are indicated by colored symbols as follows: red squares, dune FC center; blue circles, dune control; green triangles, gravel plain FC center; purple diamonds, gravel plain control.

well as by the percentages of carbon, silt, and clay (P, <0.005, <0.001, <0.001, <0.05, <0.005, and <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.

Bacterial, archaeal, and fungal richness and diversity. Analysis of the 16S rRNA gene and ITS region amplicon data sets showed that Namib Desert edaphic bacterial communities were more diverse than the fungal and archaeal communities (Table 2), with a significantly higher number of species (F was >654.5 and P was <0.001 by ANOVA), Shannon index ($F > 788.4$; $P < 0.0001$), and Simpson index ($F > 56.1$; $P < 0.001$).

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) (P, >0.05 by ANOVA), whereas fungal communities had significantly higher numbers of species in the gravel plains than in the dunes (F was >7.74 and P was <0.05 by ANOVA). 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 the zones sampled (P, >0.05 by ANOVA). The Whittaker index, measuring phylogenetic heterogeneity within zones (β-diversity), ranged from 1.52 to 1.69 for bacterial communities and from 1.40 to 1.53 for archaeal communities. Large numbers of bacterial and archaeal OTUs were also shared by all four zones (59.2% and 58.3%, respectively) (Table 2; Fig. 5a and b).

In contrast, fungal communities were highly variable in diversity, with significantly different species richness estimates across the four different zones studied (F was >7.15 and P was <0.005 by ANOVA). The dune FC soils had a significantly lower number of fungal OTUs (366 ± 59 OTUs) than all the other zones sampled (553 ± 88 OTUs for the dune control [P, <0.05 by Tukey's *post hoc* test], 613 ± 101 OTUs for the gravel plain FCs [$P < 0.01$], and 587 ± 81 OTUs for the gravel plain control [$P < 0.005$]). Fungal communities had large numbers of zone-specific OTUs (Table 2; Fig. 5c), suggesting that differential assembly processes occur for fungal communities in each of the Namib Desert soil environments studied. This is further emphasized by the fact that Whittaker indices of β-diversity, which ranged from 1.95 to 4.54, were significantly different for all zones studied (F was >17.14 and P was <0.001 by ANOVA).

Globally, *Actinobacteria* (25 to 38%), *Proteobacteria* (16 to 33%), and *Crenarchaeota* (10 to 25%) were the most abundant bacterial and archaeal phyla in all zones (see Fig. SA2a in the supplemental material), while moderately abundant taxa included *Acidobacteria* (2 to 5%), *Bacteroidetes* (3 to 20%), *Chloroflexi* (2 to 4%), *Gemmatimonadetes* (1 to 2.5%), *Verrucomicrobia* (0.9 to

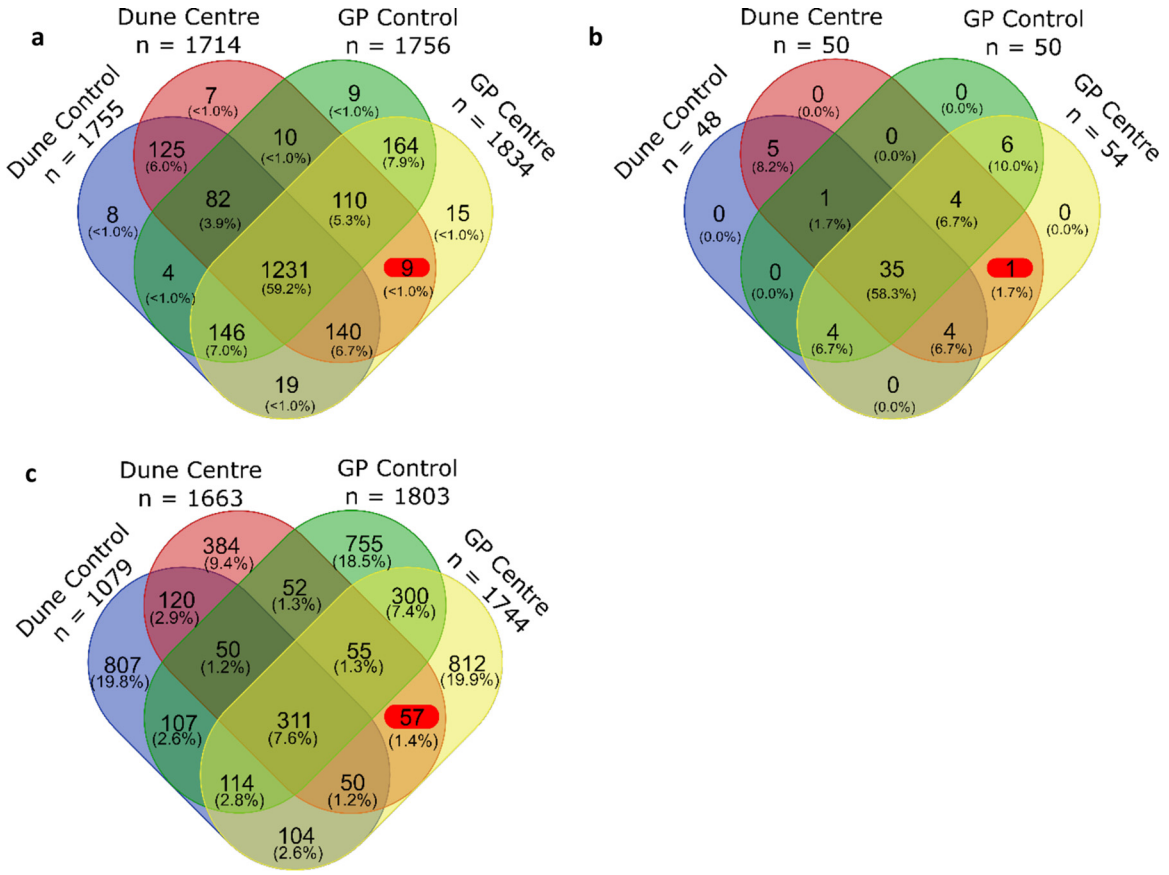

FIG 5 Venn diagrams showing the distributions of bacterial (a), archaeal (b), and fungal (c) OTUs within the soils studied. Overlap indicates the occurrence of an OTU in more than one microenvironment. The number of OTUs present solely in both the dune and the gravel plain FC centers is highlighted in red.

2.8%), and *Firmicutes* (<0.1 to 2.8%). As expected, and based on the diversity indices (Table 2), the compositions of fungal communities were highly variable between samples; each of the four zones studied was dominated by a unique fungal taxon (the class Dothideomycetes for gravel plain control soils [46.2%] and for gravel plain fairy center soils [27.5%], the class Agaricostilbomycetes for dune control soils [23.2%], and the phylum Chytridiomycota for dune fairy center soils [43.6%]) (see Fig. SA2b in the supplemental material). Globally, fungi of the phylum Chytridiomycota were significantly more abundant in dune soils than in gravel plain soils (P, <0.05 by Student's t test), while Dothideomycetes displayed the opposite trend ($P < 0.01$).

Fairy circle-specific phylotypes. We identified 9 bacterial, 1 archaeal, and 57 fungal phylotypes that were FC specific; i.e., their phylotypic signals were found only within the soils of dune and the gravel plain FCs (Fig. 5). Their phylogenetic assignments are given in Table 3. The FC-specific archaeal OTU was related to the ammonia-oxidizing genus "Candidatus *Nitrososphaera*" of the phylum *Thaumarchaeota*. The bacterial OTUs were assigned to *Cyanobacteria* (4/9), *Firmicutes* (2/9), *Proteobacteria* (2/9), and *Actinobacteria* (1/9). Although 33 of the 57 (~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. Five of the eight Chytridiomycota OTUs belonged to the genus *Rhizophlyctis*, and seven of the Ascomycota OTUs belonged to the class Dothideomycetes. Some of the OTUs assigned to Ascomycota belonged to the *Periconia*, *Phoma*, and *Curvularia* genera, and a single OTU was identified 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., in 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 of 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 the findings of 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* OTUs (recently

TABLE 3 FC-specific OTUs identified[a]

Kingdom	Phylum	Class	Order	Family	Genus	Species
Archaea	*Thaumarchaeota*	*Nitrososphaeria*	*Nitrososphaerales*	*Nitrososphaeraceae*	"*Candidatus* Nitrososphaera"	
Bacteria	*Actinobacteria*	*Nitriliruptoria*	*Nitriliruptorales*	*Nitriliruptoraceae*		
	Cyanobacteria	*Oscillatoriophycideae*	*Chroococcales*	*Xenococcaceae*		
		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				

[a] FC-specific OTUs were observed in both dune and gravel plain fairy circles. OTUs are classified to the lowest level possible using a high (>80%) level of sequence similarity.

reclassified from *Crenarchaeota* [37]) was detected in Namib Desert soils, while the crenarchaean *Thermoproteus* taxon dominated the Sonoran Desert samples (35).

Fungal diversity has not been surveyed comprehensively in desert environments worldwide (30). In the Namib Desert, fungi in mycorrhizae (38) and lichens (39) have been shown to have the potential to participate in local biogeochemical cycling, specifically by decomposing surface litter (40). Although we were able to classify only approximately 50% of the fungal OTUs (due to the underrepresentation of environmental sequences in the UNITE database [41]), this is the first study to use next-generation high-throughput sequencing to assess fungal communities within the Namib Desert. We observed that Ascomycota, Basidiomycota, and Chytridiomycota were detected ubiquitously in these desert soils. Dominant fungal genera and orders, 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 terminal restriction fragment length polymorphism (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 percentages of silt and sand. Using high-throughput sequencing, we demonstrate that the bacterial, archaeal, and fungal communities of Namib Desert dune fields and gravel plains are significantly different. We found that the percentage of sand, the pH, and the Ca content significantly shaped dune microbial (bacterial, archaeal, and fungal) communities, whereas soil P, Na, and S contents and the percentages of clay, silt, and carbon shaped the gravel plain communities. Overall, these findings support 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 for FC and control soils in either the dunes or the gravel plain, which is expected given the close proximity of each FC and control sample. Consequently, the soil properties measured could not explain the differences observed between mi-

crobial communities in FC and vegetated control soils. This suggests the potential involvement of other factors, such as other edaphic properties (e.g., Fe, Zn, and/or Al content [48]), the presence of a toxin (50), or unknown stochastic processes (51).

The four zones sampled in Namib Desert dunes and gravel plains shared large numbers of bacterial and archaeal OTUs (59.2% and 58.3%, respectively). This large set of cosmopolitan edaphic bacterial and archaeal phylotypes is indicative of a Namib Desert core community that is 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"; each zone contained a high number of unique OTUs. Fungal communities also showed a clear and significant distinction between the dune and gravel plain sites. In addition, fungal communities in FC and control vegetated soils were significantly different in composition, a pattern that was not as clearly observed with the distribution of bacterial and archaeal OTUs in the zones sampled. Because there is no significant difference between the physicochemical properties of FC and control soils, we conclude that FC-specific fungal communities are selected through habitat filtration (vegetated soil versus 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 into account the presence of fairy circles in both the gravel plain and dune environments and the differences between them. Considering that almost all FC studies have focused exclusively on dune FCs, we compared microbial communities in 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 activities (33, 54). Furthermore, microorganisms have been implicated in the formation of various FC-like phenomena, 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 of 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 data sets, respectively, that were exclusively and consistently present within dune and gravel FC soils. Of these FC-specific OTUs, many were assigned to phylogenetic groups harboring known phytotoxic or phytopathogenic microorganisms, such as the fungal genera *Periconia*, *Curvularia*, and *Aspergillus*, the fungal order Pleosporales, the fungal family Chaetomiaceae, and the bacterial class *Gammaproteobacteria* (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 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 diversity across both soil types. The high proportion 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 suggests that deterministic niche adaptation is the primary mechanism in the assembly of fungal communities (49). Finally, we have identified microorganisms that are present solely in FC soils and that may play a role in the fairy circle phenomenon. Many of the OTUs found only in FCs are related to known phytopathogen groups (the fungal genera *Periconia*, *Curvularia*, and *Aspergillus*, the fungal order Pleosporales and family Chaetomiaceae, and the bacterial class *Gammaproteobacteria* [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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