Namib Desert edaphic bacterial, fungal and archaeal communities assemble through deterministic processes but are influenced by different abiotic parameters

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Abstract

The central Namib Desert is hyperarid, where limited plant growth ensures that biogeochemical processes are largely driven by microbial populations. Recent research has shown that niche partitioning is critically involved in the assembly of Namib Desert edaphic communities. However, these studies have **mainly** focussed on the Domain Bacteria. Using microbial community fingerprinting, we compared the assembly of the bacterial, fungal and archaeal populations of microbial communities across nine soil niches from four Namib Desert soil habitats (riverbed, dune, gravel plain and salt pan). Permutational multivariate analysis of variance indicated that the nine soil niches presented significantly different physicochemistries (R^2=0.8306, P≤0.0001) and that bacterial, fungal and archaeal populations were soil niche specific (R^2≥0.64, P≤0.001). However, the abiotic drivers of community structure were Domain-specific (P<0.05), with P, clay and sand fraction, and NH_4 influencing bacterial, fungal and archaeal communities, respectively. Soil physicochemistry and soil niche explained over 50% of the variation in community structure, and communities displayed strong non-random patterns of co-occurrence. Taken together, these results demonstrate that in central Namib Desert soil microbial communities, assembly is principally driven by deterministic processes.

Keywords: Microbial community assembly; Desert ecology; Deterministic drivers; soil environments

Introduction

Arid environments represent over a third of the Earth's terrestrial surface area and are defined by an Aridity Index (AI) of below 1 (Peel et al. 2007; Pointing and Belnap 2012). These dry-land regions are poly-extreme. They are characterized by very low annual precipitation **(<250 mm)** provided by sporadic and highly unpredictable rainfall and/or fog events (Laity 2008; Lancaster et al. 1984), by large seasonal and daily temperature fluctuations (e.g. 5°C to > 45°C in the Namib Desert), by extreme ultraviolet (UV) irradiation levels (up to 100% theoretical maximum) and oligotrophy (Makhalanyane et al. 2015). In consequence, desert macro-organismal diversity is low (Heulin 2012). In contrast, edaphic microorganisms have been shown to be highly diverse, ubiquitously distributed and critical to biogeochemical cycles in hot desert systems (e.g. (Angel and Conrad 2013; Belnap 2006; Whitman et al. 1998)).

The Namib Desert spans a 2000 km longitudinal north-south zone from southern Angola to northern South Africa, and stretches 150-200 km inland from the western coast (Eckardt et al. 2013). It is thought to be the oldest dryland environment on Earth, with an estimated age of 43 million years and with the central section being hyper-arid (AI < 0.05) for the last 5 million years (Seely and Pallett 2008). The Namib Desert is characterized by a particular east-west inverse rain/fog aridity gradient, with annual precipitation increasing from the western coast (10 mm per annum; mainly fog) towards its eastern boundary, the Great Escarpment (60 mm per annum; mainly rain; (Eckardt et al. 2013; Olivier 1995). It also comprises a variety of soil habitats: the expansive gravel plains north of the Kuiseb river, in which several salt pans (or playas), inselbergs and ephemeral riverbeds occur, and the sand dunes of the Namib Sand Sea south of the Kuiseb river (Seely and Pallett 2008).

Edaphic microbial communities play a significant role in ecosystem productivity, and, in the absence of plants, contribute to the majority of carbon, nitrogen and phosphorus cycling (Allison and Martiny 2008; Makhalanyane et al. 2015; Prosser and Nicol 2012). The assembly of these communities depends largely on four processes; diversification, dispersal, drift and selection (Vellend 2010). Diversification, dispersal and drift are considered to be stochastic processes, while selection (or niche partitioning) is a deterministic process (Nemergut et al. 2013). Niche partitioning involves the selection of microbial taxa capable of colonizing an environment based on its abiotic characteristics. It follows that highly similar environments will select for similar microbial communities (Nemergut et al. 2013; Vellend 2010).

In soil ecosystems, including desert soils, it has been repeatedly demonstrated that soil physicochemical properties, climate and geographic spatial scales affect microbial community assemblages (e.g., (Fierer et al. 2012; Gombeer et al. 2015; Lalley et al. 2006; Ronca et al. 2015; Stomeo et al. 2013). In the Namib Desert, we have conclusively demonstrated that niche partitioning/selection is a critical process involved in the assembly of the bacterial fraction of edaphic and hypolithic microbial communities (Gombeer et al. 2015; Makhalanyane et al. 2013; Ramond et al. 2014; Ronca et al. 2015). Most similar research has focussed on the Domain Bacteria, with few multi-domain studies which include **Fungi** (Ramond et al. 2014), protists (Valverde et al. 2015), viruses (Prestel et al. 2008), **and/or Archaea** (Van der Walt et al. 2016). Since Bacteria, Fungi and Archaea are involved in distinct functional aspects of ecosystem processes and biogeochemical cycling (e.g. methanogenesis by Archaea, organic matter decomposition by fungi, photosynthetic carbon fixation by cyanobacteria (Crowther et al. 2014; Prosser and Nicol 2012), **we suggest that** these groups should be studied together. Such a holistic approach would allow a better understanding of how members from these domains interact, as well as clarifying their respective role(s) in ecosystem service delivery (Leininger et al. 2006; Waldrop and Firestone 2006).

We therefore analysed bacterial, archaeal and fungal community fingerprints (using Terminal-Restriction Fragment Length Polymorphism [T-RFLP]) from various Namib Desert soil **types (n = 36) and compared their structure and assembly. We examined whether shifts in microbial community structure were related to deterministic or stochastic effects (Nemergut et al. 2013).** We hypothesised that community assembly should differ between the respective domains, with (i) the bacterial and archaeal communities shaped by deterministic effects (Angel et al. 2010) while (ii) the fungal communities rather by stochastic processes (Powell and Bennett 2016). **Previous studies in the Namib Desert have indeed shown that deterministic processes influenced edaphic bacterial communities (Gombeer et al. 2015; Ramond et al. 2014; Stomeo et al. 2013); while edaphic fungal communities, which are more resilient to extreme environmental conditions (Barnard et al. 2013), are known to be influenced by stochastic factors (Beck et al. 2015; Powell and Bennett 2016).**

Materials and Methods

Sample Collection

Fig. 1. Map displaying the Central Namib Desert sampling sites studied. The River Bed consisted of a wet (RBW) and dry (RBD) portion of the Kuiseb river, while the Dune samples consisted of dune top (DT), dune slope (DS) and interdune (ID) samples of the Kahani dune system. Salt pan samples consisted of the wet (SPW) and dry (SPD) portion of the Hosabes saline spring. Hyperarid grave plain (HAGP) and arid gravel plain (AGP) sampling sites are also indicated. Image produced using Google Earth, © 2016 DigitalGlobe

Surface soil (0-3cm, **450-500g**) samples from nine edaphic environments (niches) were collected in April 2014 in the central Namib Desert, Namibia (Figure 1; Supplementary Figure 1). Gravel plain soil samples were collected from hyperarid (**HAGP**, n=4, 100 km inland, 23°21'45.53"S; 15°23'2.37"E) and arid (**AGP**, n=4, 190 km inland, 23°17'50.46"S; 16° 4'35.40"E) zones of the gravel plains **(Eckardt et al. 2013). Soil from the stream (SPW, n=4) and the adjacent dry (SPD, n=4) soils were sampled** from the source and sink of the Hosabes salt pan (23°30'25.53"S; 15°4'17.98"E) (Adriaenssens et al. 2016). Dune soils from the top (**DT,** n=4), slope (**DS,** n=4) and interdune (**ID,** n=4) sections of the Kahani dune system adjacent to the Gobabeb Research and Training Centre were collected (Ronca et al. 2015). Finally, Kuiseb riverbed soils (23°33'44.65"S; 15°2'11.11"E) were harvested from wet (**RBW,** n=4) and dry (**RBD,** n=4) portions 3 days after the river flooded (Frossard et al. 2015). Four replicates **per soil niche, located 50 m apart, were aseptically collected from vegetation-free patches**, resulting in a total of 36 individual soil samples. These were kept at room temperature for transport to the University of Pretoria, where they were stored at -20°C until subsequent molecular analysis.

Soil physicochemistry analyses

Fourteen soil physicochemical properties were analysed for all samples. Soil pH was averaged from triplicate measurements of soil slurries (4g soil in 10ml deionised water) with a pH meter (Crison Basic +20, LasecSA). Total carbon percentage was determined by potassium dichromate and sulfuric acid oxidation and subsequent titration of excess dichromate (Nelson and Sommers 1996). Total ammonium and nitrate concentrations were quantified by potassium chloride extraction and steam distillation followed by titration (Keeney and Nelson 1982). Phosphorus levels were determined by the Bray I method (Bray and Kurtz 1945), and ion concentrations (calcium, sodium, potassium and magnesium) by ammonium acetate extraction followed by inductively coupled plasma atomic emission spectroscopy (Rhoades 1982). Sand, silt and clay fractions were determined by hydrometer readings (Bouyoucos 1962). **Soil moisture content was determined gravimetrically by drying 25g of soil at 105°C for 12 hours (Gombeer et al. 2015).**

Metagenomic DNA extraction, PCR amplification and T-RFLP fingerprinting

Soil metagenomic DNA (mDNA) was extracted using a modified phenol-chloroform extraction (Gunnigle et al. 2014). In brief, 250 mg of 0.1 mm and 0.5 mm zirconia beads were added to sterile 2 ml screw cap tubes, followed by the addition of 1 g of soil.

Thereafter, 500 µl extraction buffer (5% w/v CTAB, 0.7 M NaCl and 240 mM KH_2PO_4, pH 8.0) and 500 µl phenol:chloroform:isoamyl alcohol (25:24:1 v/v) was added to each tube. Up to five extractions were pooled before precipitation with polyethylene glycol (2:1 volume of 30% w/v PEG 6000, 1.7 M NaCl) to extract sufficient mDNA for downstream analysis. The quantity and quality of the extracted mDNA was assessed using a NanoDrop Spectrophotometer and agarose gel electrophoresis on 1% (w/v) agarose gel.

Bacterial 16S rRNA gene amplicons were generated using the FAM-labelled 341F (5'-CCTACGGGAGGCAGCAG-3') and 908R (5'- CCGTCAATTCMTTTGAGTTT-3') primer pair (Ishii and Fukui 2001; Lane et al. 1985) and the archaeal marker with the FAM-labelled 340F (5`-CCCTAGGGGYGCASCAG-3`) and 1000R (5`-GGCCATGCACYWCYTCTC-3`) primer pair (Gantner et al. 2011). Fungal internally transcribed spacer (ITS) amplicons were generated using the FAM-labelled ITS1F (5`-CTTGGTCATTTAGAGGAAGTAA-3`) and ITS4 (5`-TCCTCCGCTTATTGATATGC-3`) primer pair (Martin and Rygiewicz 2005). For bacterial and fungal phylogenetic marker amplifications, 25 µl PCR reactions containing 1X DreamTaq reaction buffer (Thermo Scientific), 0.2 mM dNTPs, 0.65 U DreamTaq (Thermo Scientific), 0.1 mg/ml bovine serum albumin, 0.5 µM forward primer and 1 µM reverse primer were prepared. For archaeal 16S rRNA gene amplifications, 25 µl PCR reactions contained 1X KAPA Robust Buffer A (KAPA Biosciences), 0.2 mM dNTPs, 1 X Enhancer A (KAPA Biosciences), 0.5 µM forward primer and 1 µM reverse primer and 1 U KAPA Robust Taq DNA polymerase. An estimated 20 ng of mDNA was used for PCR.

For T-RFLP, triplicate PCR reactions were pooled and purified using a GeneJET Gel Extraction and DNA Clean-up Micro Kit (Thermo Scientific) according to the manufacturer's instructions. The concentration of the resulting purified PCR amplicons was determined spectrophotometrically using a Peqlab ND-1000 NanoDrop Spectrophotometer. 200 ng (bacterial and archaeal) and 400 ng (fungal ITS) of purified PCR amplicons were subjected to *Hae*III digestion (Ramond et al. 2014). ***Hae*III was used as it has previously been shown to retrieve a high number of terminal restriction fragments (T-RFs) when compared to other restriction enzymes when studying Namib Desert edaphic communities** (Gombeer et al. 2015; Ramond et al. 2014). Digested PCR amplicons were purified using a GeneJET Gel Extraction and DNA Cleanup Micro Kit (Thermo Scientific). A total of 4 µl of the purified product was added to 6.75 µl deionised formamide and 0.25 µl ABI GeneScan LIZ-600 Size Standard V2.0 in a 96-well plate, and the resulting plate was subjected to fragment analysis on an Applied

Biosystems 3500XL genetic analyser (SeqServe, University of Pretoria, http://seqserve.bi.up.ac.za/). The resulting terminal restriction profiles were filtered and binned using the R and Perl software packages, and one T-RF was equated to one operational taxonomic unit (OTU) (Abdo et al. 2006; R Core Development Team 2015).

Statistical Analysis

Soil physicochemical properties were analysed using the ggbiplot, FactoMineR, lattice, gplots, MASS and vegan packages in R (Dixon 2003; Gregory R. Warnes 2015; Le et al. 2008; Vu 2015). Based on quantile-quantile comparison plots, selected variables (Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4, NO_3, P **and soil moisture content**) were log-transformed and the resulting matrix was used to compute Euclidean distances and subjected to principal component analysis (PCA). Within-group variances was assessed using the betadisper function in R. Permutational multivariate analysis of variance (PERMANOVA) was conducted to identify significant differences between soil environments and soil niches based on physicochemical data. Tukey's Honest Significant Difference was computed to identify pairwise differences in soil type physicochemistries.

Relative abundances of T-RFLP peaks were averaged from three technical replicates, and the resulting matrix was analysed with R (R Core Development Team 2015). For the analysis of individual domains (Bacteria, Fungi or Archaea), T-RFLP relative abundance tables were Hellinger-transformed (Legendre and Gallagher 2001). Bray-Curtis dissimilarity matrices were computed and non-metric multidimensional scaling (NMDS) plots generated (Bray and Curtis 1957; Clarke 1993). PERMANOVA was performed on the respective community matrix to evaluate if microbial communities were significantly different in the soil niches studied. A combination of soil physicochemical data and community matrices were used in a redundancy analysis (RDA) to visualise the effects of soil physicochemical properties on the structure of soil microbial communities using the vegan package in R. Only significant soil physicochemical parameters, as identified by an ENVFIT analysis, were plotted on the resulting RDA plot. Variation partitioning was conducted in vegan as described elsewhere (Caruso et al. 2011). Co-occurrence null model analysis, based on community presence/absence data, was conducted using the EcoSimR package in R, as described by Stomeo (Stomeo et al. 2013).

Results

Soil physicochemical analysis

The PCA plot (Figure 2) based on the 13 soil physicochemical properties measured explained **69.3% of the variation among samples (50.6% for axis 1 and 18.7% for axis 2)**. Overall, the soils studied were alkaline (pH 8.4-9.5, Table 1). The different soils resolved into three separate clusters on the PCA plot: Cluster 1 consisted of dune (DT, DS and ID) and RBD soils; Cluster 2 of the salt pan soils (SPD and SPW) and Cluster 3 of gravel plain (AGP and HAGP) and RBW soils (Figure 2). Cluster 1 soils mainly separated from the other soils based on their significantly higher proportion of sand ($P<0.05$, Figure 2, Table 1, Supplementary Table 1) and the salt pan soils (SPW and SPD) of cluster 2 separated due to their high ion concentrations (particularly nitrate, Na^+, K^+, Ca^{2+} and Mg^{2+}) **and higher soil moisture content**. Interestingly, within cluster 2, salt pan dry soils separated from the wet soils as they contained globally higher levels of both the measured ions and percentage silt ($P<0.05$, Table 1, Figure 2, Supplementary Table 1). Cluster 3 soils were globally characterized by elevated CEC, P and NH_4^+ contents, and carbon and clay percentages. Within this cluster, AGP and HAGP samples formed non-overlapping sub-clusters: HAGP soils were characterised by significantly higher levels of NH_4^+, NO_3^-, Ca^{2+} and Na^+ (Table 1, Supplementary Table 1), AGP by higher P content and CEC, and the RBW soils by higher total carbon content (Table 1). A multivariate homogeneity of group dispersions analysis (betadisper) indicated that the within-group variances of the soil niches were not significantly different (Tukey's Honest Significant Difference $P > 0.1$). Furthermore, based on PERMANOVA, the four soil habitats ($R^2=0.568$, pseudo-$F=13.152$, $P\leq0.001$) and the nine soil niches ($R^2=0.8306$, pseudo-$F=15.322$, $P\leq0.0001$) presented significantly different soil physicochemistries.

Diversity and structure of central Namib Desert edaphic microbial communities

Metagenomic DNA was successfully recovered from all the soil samples, with the exception of two of the four RBD soils. Bacterial OTUs were ubiquitously detected, while fungal OTUs were absent from dune tops (DT) and slopes (DS), and archaeal OTUs from dune tops (DT) and slopes (DS) and riverbed soils (RBD and RBW, Table 2). In total, 300 unique microbial OTUs were identified; 60 bacterial, 147 fungal and 93 archaeal OTUs respectively. OTU richness per soil niche ranged from 3 (ID) to 25 (HAGP) bacterial OTUs, from 25 (SPW and RBD) to 61 (AGP) fungal OTUs and from 16 (ID) to 48 (AGP) archaeal OTUs (Table 2). Overall, gravel plain microbial communities were the richest in terms of both total OTUs and OTUs per sample (Table 2) and contained the highest proportion of unique OTUs (36%, 108/300), followed by the salt pan (15%, 45/300), dune (9.33%, 26/300) and riverbed (6%, 18/300) communities. Interestingly, in SPW soils, the archaeal

Table 1. Soil physicochemical properties of the nine soil niches included in this study. Values are given as the mean of four replicates ± standard deviation. ND: Not detected.

Soil Chemistry	Gravel Plain		Dune			Salt Pan		River Bed	
	HAGP	AGP	DT	DS	ID	SPW	SPD	RBD	RBW
pH	9.5 ± 1.3	8.8 ± 0.9	8.8 ± 0.2	8.4 ± 0.1	8.5 ± 0.0	8.6 ± 0.5	8.8 ± 0.6	8.7 ± 0.04	8.5 ± 0.3
CEC (cmol/kg)	21.9 ± 3.5	25.9 ± 3.5	8.5 ± 1.2	9.6 ± 1.6	8.5 ± 1.2	13.0 ± 2.4	6.7 ± 0.2	6.6 ± 0	23.8 ± 10.5
Carbon (%)	0.3 ± 0.1	0.7 ± 0.3	0.02 ± 0.01	0.03 ± 0.01	0.09 ± 0.03	0.6 ± 0.2	0.7 ± 0.5	0.2 ± 0.07	1.3 ± 0.4
NH_4 (mg/kg)	34.9 ± 22.1	3.5 ± 0.4	5.9 ± 0.7	6.3 ± 1.9	5.5 ± 1.2	5.6 ± 1.4	5.5 ± 1.7	3.5 ± 0.7	32.7 ± 23.8
NO_3 (mg/kg)	30.8 ± 21.8	1.4 ± 0.2	4.8 ± 0.7	5.5 ± 1.9	7.6 ± 1.9	4.0 ± 1.8	52 ± 28.9	1.5 ± 0.03	25.5 ± 13.1
Sand (%)	80.0 ± 6.8	78.8 ± 3.0	100.0 ± 0	100.00 ± 0	99.0 ± 0.0	87.8 ± 7.1	73.9 ± 11.6	99.3 ± 0.1	65.4 ± 10.2
Silt (%)	8.5 ± 7.9	7.7 ± 2.0	ND	ND	1 ± 0	10.5 ± 1	20 ± 4.32	1 ± 0	10.5 ± 1.9
Clay (%)	11.5 ± 3	13.5 ± 3	ND	ND	ND	1.7 ± 6.1	6.1 ± 8.7	ND	24.1 ± 8.4
K (mg/kg)	133.3 ± 31.6	75.3 ± 19.9	16.4 ± 3.1	24.8 ± 1.6	23.9 ± 1.5	599.8 ± 271.9	1014.2 ± 411.6	64.1 ± 16.4	250.3 ± 140.4
Ca (mg/kg)	4593.0 ± 301.0	1013.8 ± 526.3	9.9 ± 2.9	47.1 ± 16.9	11.9 ± 1.4	17084.2 ± 10771.4	34126.7 ± 1437.8	889.3 ± 125.4	4631.7 ± 2547.0
Na (mg/kg)	23.7 ± 15.2	0.05 ± 0.04	8.5 ± 4.0	7.3 ± 0.9	18.5 ± 1.6	7530.7 ± 3349.5	21600.2 ± 8688.2	27.6 ± 1.5	127.0 ± 32.9
Mg (mg/kg)	59.8 ± 9.5	53.1 ± 16.0	1.3 ± 0.4	4.1 ± 0.7	1.7 ± 0.2	451.5 ± 170.1	694.6 ± 150.5	30.9 ± 3.1	285.8 ± 154.5
P (mg/kg)	2.1 ± 0.3	28.3 ± 6.0	0.6 ± 0.2	1.4 ± 0.1	0.4 ± 0.1	2.2 ± 2.8	4.1 ± 4.4	3.4 ± 1.0	4.9 ± 4.8
Soil Moisture (% w/w)	0.84 ± 0.09	1.05 ± 0.34	0.31 ± 0.11	0.22 ± 0.1	0.39 ± 0.04	Saturated	23.37 ± 3.59	19.97 ± 7.21	0.19 ± 0.01

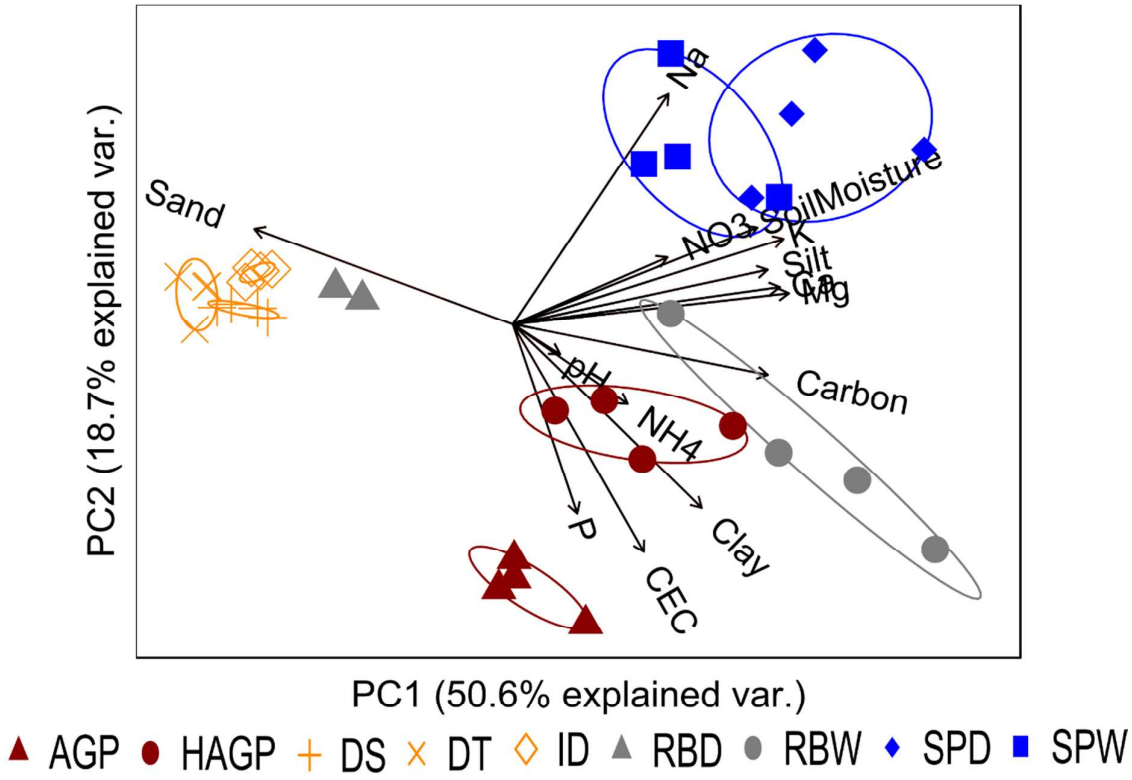

Fig. 2. Principal component analysis biplot of Euclidean distances based on soil physicochemical parameters. Ellipses group samples according to their soil niche of origin (n =9). Vectors indicate proportion (length) and direction (orientation) of variable gradients on the ordination

community was much more diverse than the fungal or bacterial communities (Table 2). Additionally, in ID soil samples, which is the only dune soil niche which contained OTUs from all three Domains, the fungal community was the most diverse (Table 2). Fungal diversity was also much higher in RBW samples in comparison to RBD soils (Table 2). Furthermore, only 3.3% (2/60) of bacterial, 7.4% (11/147) of fungal and 8.6% (8/93) of archaeal OTUs presented a cosmopolitan distribution, i.e., occurred in all the soil types studied (Supplementary Figure 2).

Non-metric multidimensional scaling (NMDS) plots were used to assess the structures of the dominant bacterial, fungal, and archaeal assemblages (Figure 3). Globally, populations from each soil niche clustered together in the plots, which indicated significant structural homogeneity. This was confirmed by betadisper analyses of β-diversity, which revealed that variances within soil niches were not significantly different ($P = 0.88$). On the NMDS plot, bacterial assemblages formed four distinct clusters (Figure 3A): The AGP, HAGP and SPW bacterial populations formed individual clusters, and the remaining populations (i.e., DS, DT, ID, SPD, RBD and RDW) clustered together. Fungal assemblages formed four other clusters (Figure 3B; cluster 1: AGP and SPW, cluster 2: HAGP and RBW, cluster 3: SPD and RBD, and cluster 4: ID) and the archaeal assemblages five (Figure 3C; cluster 1: AGP, cluster 2: HAGP, cluster 3: ID, cluster 4: SPW and cluster 5: SPD). However, PERMANOVA analyses revealed that the bacterial (R^2=0.75, pseudo-F= 9.33, $P \leq 0.001$), fungal (n=7, R^2=0.70, pseudo-F= 7.83, $P \leq 0.001$) and archaeal (n=5, R^2= 0.64, pseudo-F=6.58, $P \leq 0.001$) populations were soil niche-specific.

Redundancy and ENVFIT analyses were used to identify the environmental drivers that significantly influenced Namib Desert edaphic community assemblies (Figure 4). The RDA plots strongly resemble the NMDS plots (Figure 3). AGP bacterial populations were shaped by P and CEC, while Ca^{2+}, Mg^{2+} and K^{+} were the main abiotic drivers of HAGP and SPW bacterial populations (Figure 4A). **Additionally, SPW communities were also shaped by the higher moisture content of this niche**. The bacterial populations of the remaining niches formed a dense cluster which was not resolved by specific abiotic parameters. Fungal population structures in RBW and AGP soils were shaped by NH_4^{+}, CEC, percentage carbon and clay fraction, whereas the percentage sand was the main driver of ID, SPD and RBD fungal populations (Figure 4B). Archaeal populations in gravel plain soils (AGP and HAGP) were shaped CEC and NH_4^{+}, whereas salt pan (SPW and SPD) archaeal assemblages were strongly shaped by ion concentration (Na^{+}, Ca^{2+}, Mg^{2+} and K^{+}), percentage carbon, silt fraction **and soil moisture content** (Figure 4C).

Table 2. Mean alpha diversity, beta diversity (Whittaker) and gamma diversity of bacterial, fungal and archaeal communities from the nine respective soil niches. ND: No T-RFs were detected.

Domain		Gravel Plain		Dune			Salt Pan		River Bed	
		AGP	HAGP	DT	DS	ID	SPD	SPW	RBD	RBW
Bacteria	Alpha	15±0.81	13.50±2.38	3±0.82	4.25±1.90	1.750±0.5	5±1.63	8.75±2.5	6.50±0.7	1.75±0.95
	Beta	1.34	1.85	2	2.82	1.71	2.4	1.94	1.69	2.29
	Gamma	20	25	6	12	3	12	17	11	4
Fungi	Alpha	23.75±10.63	21.50±3.79	ND	ND	26.50±6.35	19±2.58	17.25±3.30	17.67±2.52	22.50±6.29
	Beta	2.57	2.37	ND	ND	1.81	1.58	1.45	1.42	1.98
	Gamma	61	51	ND	ND	48	30	25	25	44
Archaea	Alpha	23.25±1.89	19.75±4.35	ND	ND	10.75±2.22	8.25±0.96	14.75±9.32	ND	ND
	Beta	2.06	2.13	ND	ND	1.49	1.56	2.58	ND	ND
	Gamma	48	42	ND	ND	16	13	38	ND	ND

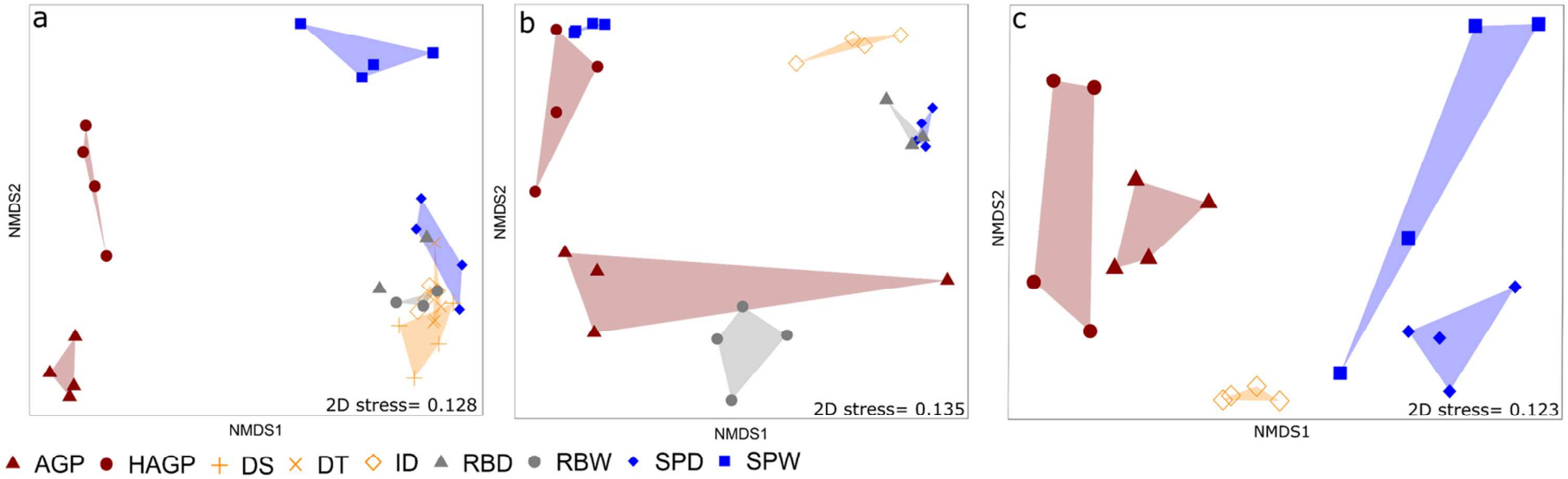

Fig. 3. NMDS ordination plots of Hellinger-transformed Bray–Curtis dissimilarity matrices of the OTU relative abundances in the bacterial (a), fungal (b) and archaeal (c) communities. Hulls link individual samples from the same soil niche

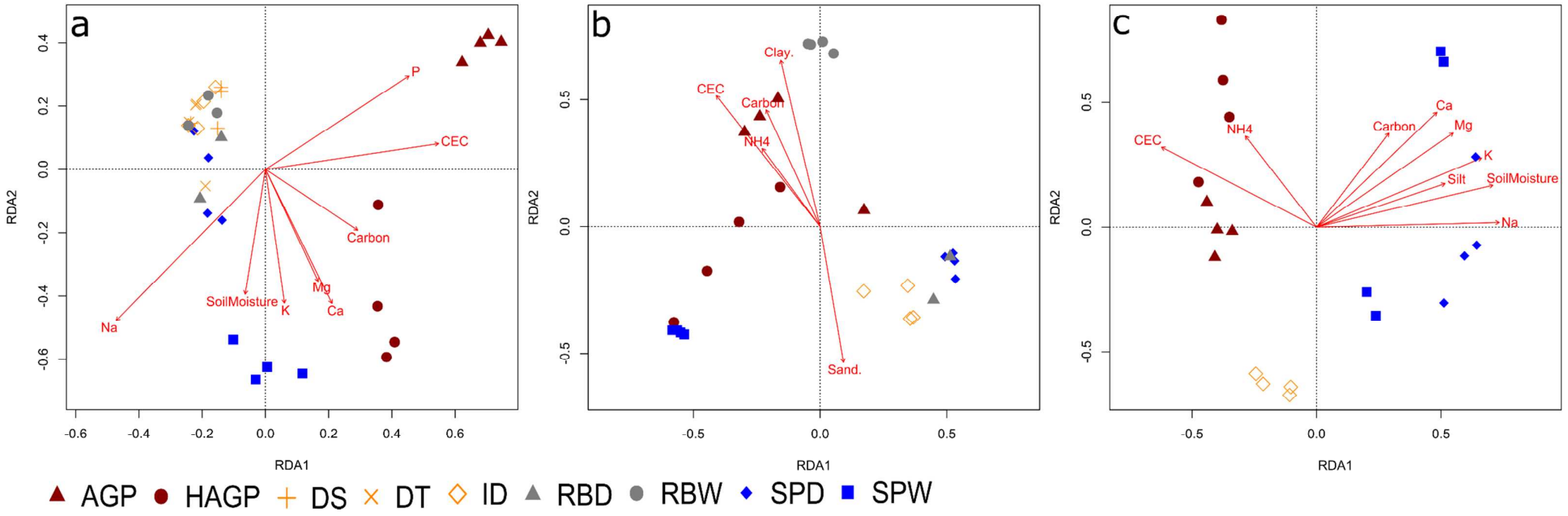

Fig. 4. Redundancy analysis (RDA) plots of central Namib Desert bacterial (a), fungal (b), archaeal (c) communities and the response of these communities to significant soil physicochemical properties. Vectors, indicated by red arrows, indicate proportion (length) and direction (orientation) of variable influences on the ordination

Table 3. Variation partitioning of the structure of Domains Bacteria, Fungi and Archaea into soil physicochemistry [E] and soil niche [S] and the overlap between these components [ES] using redundancy analysis

Domain	Bacteria	Fungi	Archaea
Total Explained Variation (R^2)	0.58	0.64	0.64
Environmental Effect [E]	0.03	0.10	0.14
Soil Niche[S]	0.10	0.22	0.13
Overlap of E and S [ES]	0.44	0.29	0.37
Unexplained Variation	0.42	0.36	0.36
P-value	≤ 0.001	≤ 0.001	0.035

Table 4. Results from co-occurrence null model analysis. 1000 randomly generated tables were compared to the respective observed community matrix. The c-score metric was used for the analysis.

Domain	Observed C-score	Mean Simulated C-score	Standardized Effect Size	P-value
Bacteria	5.8497	11.324	-7.3827	< 0.001
Fungi	7.3241	10.326	-6.9352	< 0.001
Archaea	5.3202	7.2703	-4.7574	< 0.001

Variation partitioning was used to determine how much of the variation in community structure could be attributed to soil niche [S] or **the fourteen soil physicochemical (environmental) variables [E]**, and the overlap between these constraining variables [ES]. The resulting models, based on RDA, indicated that the combination of spatial (i.e., soil niche) and environmental (i.e., soil physicochemistry) effects accounted for more than 50% of the variation of all the communities studied (**Table 3; i.e., bacterial [58%], fungal [64%], and archaeal [64%] communities**). These results strongly suggested that niche partitioning is critical in the assembly of Namib Desert soil microbial communities. **Spatial scale accounted for ~34.4% (0.22/0.64) of the proportion of variation for the assembly of the fungal assemblages, but only 17.2% (0.1/0.58) and 20.3% (0.13/0.64)** for the bacterial and archaeal assemblages, respectively. To evaluate the importance of stochasticity in community assembly, null model analysis was used, which compared the observed patterns of species co-occurrence with randomly generated communities (based on the C-score metric (Etienne 2009)). The null model was rejected ($P<0.001$); i.e., the patterns in species co-occurrence were non-random, as shown by the large and negative standardized effect sizes (SES) for bacterial (-7.38), fungal (-6.94) and archaeal (-4.76) populations (Table 4). Consequently, these communities are clearly segregated based on their specific soil niche, i.e., shaped by deterministic processes.

Discussion

We used molecular T-RFLP fingerprinting to compare the bacterial, fungal and archaeal community structures and assemblies in nine contrasted central Namib Desert soil niches from four terrestrial habitats. While we acknowledge that molecular fingerprinting techniques such as T-RFLP only reflect the dominant phylotypes in the population, we note that duel restrictions have been shown to be as effective as next generation sequencing in resolving environmental and ecological patterns of microbial communities (Gobet et al. 2014; Powell et al. 2015; van Dorst et al. 2014). Additionally, in desert environments where microbial diversity is limited, at least in comparison to more productive ecosystems (Caruso et al. 2011; Fierer et al. 2012; Makhalanyane et al. 2015), **the use of T-RFLP has resulted in ecologically coherent patterns when evaluating the structures and assemblies of microbial communities** (e.g., Angel et al. 2010; Frossard et al. 2015; Gombeer et al. 2015; Makhalanyane et al. 2013; Ramond et al. 2014; Stomeo et al. 2013).

Globally, edaphic bacterial and archaeal assemblages are shaped mainly by deterministic factors, such as soil **pH (Fierer and Jackson 2006)** and precipitation (Angel et al. 2010),

while fungal populations are rather more influenced by stochastic processes (Beck et al. 2015; Powell and Bennett 2016). In deserts, including the Namib Desert, abiotic factors and environmental conditions are critical in shaping edaphic microbial communities, and niche-partitioning has been found to profoundly influence edaphic and hypolithic bacterial community assembly (Andrew et al. 2012; Bates et al. 2012; Gombeer et al. 2015; Makhalanyane et al. 2013; Ramond et al. 2014; Ronca et al. 2015; Stomeo et al. 2013). Similarly, by using T-RFLP and next generation sequencing methods, fungal communities were found to be influenced by their edaphic environment (Frossard et al. 2015; Ramond et al. 2014; Van der Walt et al. 2016). The only study to date assessing Namib Desert archaeal communities (Van der Walt et al. 2016) found that archaeal populations were significantly different in two distinct edaphic environments, namely gravel plain and dune soils.

However, all these studies focused either on a single domain (Gombeer et al. 2015; Ronca et al. 2015) or compared only two edaphic habitats (Frossard et al. 2015; Ramond et al. 2014; Van der Walt et al. 2016). In this study, we have compared the archaeal, bacterial and fungal assemblages of four distinct Namib Desert soil habitats (dune, gravel plain, riverbed and saltpans), subdivided into nine soil niches. Such a holistic approach was designed to fully assess the assembly of desert microbial communities and thus better understand their role in providing vital ecosystem functions (Isobe and Ohte 2014; Leininger et al. 2006). It has recently been shown that, when microbial diversity and assembly mechanisms are included in ecosystem turnover models, their functional rates estimates are significantly improved (Graham et al. 2016).

Physicochemistry of central Namib Desert soil environments

The central Namib Desert is composed of a diverse range of soil environments (Gombeer et al. 2015). In this study, based on their physicochemistries, the four soil habitats and nine soil niches studied only separated into three distinct groups. The dune (DT, DS and ID) and dry river bed (RBD) soils comprised of homogenous physicochemical characteristics (Gombeer et al. 2015; Ronca et al. 2015), as (i) dune soils are homogenised through aeolian activity (Laity 2008; Livingstone 2013) and (ii) the Kuiseb River, which separates the Namib Sand Sea from the gravel plains, can be the receptacle of dune sand through wind dispersal and deposition (Livingstone 2013). However, when wet, physicochemical composition of riverbed soils (RBW) is closely related to those of the gravel plain soils (AGP and HAGP). This group of samples was characterized by high CEC, ammonium, nitrate and total carbon levels. The physicochemical characteristics of the wet portion of

the river bed soils was likely altered by water flow, which resulted in the deposition and concentration of smaller particles (such as clay and silt (Malmon et al. 2004)), organic matter (Wagener et al. 1998) and other nutrients (phosphorus and nitrate (Jones et al. 2015)). Salt pan soils (SPD and SPW), which contained higher levels of ions **and elevated soil moisture content**, formed the third distinct cluster. This was also expected as the high evaporation rate of groundwater from these saline springs lead to the concentration of Na^+, Ca^{2+}, Mg^{2+} and K^+ ions (Day and Seely 1988). We also observed that the dry and wet portions of the saltpans presented different characteristics. This is due to the fact that the crust surrounding the saline spring is mainly composed of gypsum and halite, resulting in high levels of salinity and ion concentrations (Day and Seely 1988).

However, the nine respective soil niches ultimately represent significantly different soil physicochemical signatures. This strong overall environmental dissimilarity thus represented an excellent environment to test hypotheses on the structures and assemblies of desert edaphic microbial communities (Ramette 2007).

Drivers of Namib Desert microbial community assembly

In soil ecosystems, multiple environmental factors have been shown to significantly affect their indigenous edaphic communities. For example, water content (Clark et al. 2009), micronutrients such as P and N (Preem et al. 2012), percentage soil carbon (Kuramae et al. 2012), soil pH and particle size (Lauber et al. 2008), and soil salinity (Sardinha et al. 2003) were found to be significant environmental drivers of the assembly of microbial populations. Here, we demonstrate that Namib Desert bacterial, fungal and archaeal communities were driven by distinct edaphic drivers (Angel et al. 2010; Powell et al. 2015).

Our results indicated that the dominant bacterial and archaeal population structures were significantly shaped by ion concentrations **and soil moisture content**, whereas fungal population structure was strongly influenced by soil structure. The three Domains also shared universal drivers, notably CEC and total carbon content. However, the influence of these drivers were soil niche-specific: Percentage carbon significantly shaped bacterial populations in HAGP soils, whereas it significantly shaped AGP and RBW fungal and salt pan (SPW and SPD) archaeal population structure. Furthermore, as previously observed, ion concentrations had a more pronounced effect on prokaryotic (Bacterial and Archaeal) population structure than fungal populations (Sardinha et al. 2003). In gravel plain soils, the higher CEC and P levels, which is indicative of more fertile soil (Davatgar et al. 2012), might explain the highly diverse bacterial, archaeal and fungal assemblages observed, as

the higher abundance of resources could support a more diverse community of microorganisms. **This higher nutrient content is likely due to mineral deposits, particularly subsurface gypsum and $CaCO_3$ found in gravel plain soils (Gombeer et al. 2015). For HAGP samples specifically, oceanic fog likely constitutes the source of their higher levels of Ca^{2+}, ammonium and nitrate (Eckardt et al. 2013; Olivier 1995). Additionally, the higher moisture content of salt pan soils significantly affected their bacterial and archaeal community structures. Fungi are generally described as more resilient to the effects of wetting and drying of soils, whereas bacteria are more sensitive to wetting and drying events (Angel and Conrad 2013; Barnard et al. 2013; Waldrop and Firestone 2006). However, the combination of higher salinity, in addition to soil moisture content were drivers of bacterial and archaeal community structure in salt pan soils.**

The ubiquitous presence of bacteria in all nine soil niches was not surprising, as bacteria are well adapted to survive in extreme edaphic environments, and have consistently been detected across all Namib Desert edaphic environments (Gombeer et al. 2015; Makhalanyane et al. 2013; Prestel et al. 2008; Ramond et al. 2014; Ronca et al. 2015; Stomeo et al. 2013; Valverde et al. 2015; Van der Walt et al. 2016).The absence of Archaea and Fungi from dune top and dune slope soils could reflect the highly unstable nature of the soil in this environment, which has a high dispersal rate of soil particles (Livingstone 2013). However, the possibility that the limitations of techniques used in this study resulted in the absence of Archaeal and Fungal OTUs should not be eliminated, as T-RFLP analysis is only able to detect the most abundant phylotypes present (Nocker et al. 2007).

Phosphorus content has been shown to impact microbial community structure and function (Bell et al. 2009). However, our results suggested that P content only significantly influenced bacterial community structure. Fungal populations in the wet portion of the riverbed, with elevated carbon levels, seem to be more adapted to colonising this soil niche, as evidenced by the increase in fungal diversity from RBD to RBW soils, while the opposite trend is seen for bacteria. In comparison to bacteria, fungal communities are capable of degrading a wider variety of carbon sources and other sources of organic matter, which could explain their dominance in this soil niche (Kuzyakov 2010). Furthermore, the growth of fungal hyphae is determined largely by soil particle size (Gupta and Germida 2015), which can explain the strong correlations of Namib Desert fungal population structures with this abiotic parameter. Moreover, Archaea are known to be

adapted to extremely saline environments (Roeßler and Müller 2001; Sardinha et al. 2003), which likely resulted in higher diversity and unique structure of archaeal populations in salt pan soils, in comparison to bacterial populations in the same soil niche (Roeßler and Müller 2001; Valentine 2007).

The assembly of environmental communities is governed by four processes, namely selection, drift, speciation and dispersal (Vellend 2010). The fact that we observed bacterial, fungal and archaeal assemblages that were specific to different Namib Desert soil niches, is strongly suggestive that deterministic processes **(habitat-filtration and niche-partitioning)** are important in their assembly (Martiny et al. 2006; Nemergut et al. 2013). **This is further confirmed by the fact that only a limited number of OTUs were cosmopolitan. Such patterns of community composition and structure are indicative of strong selective pressures occurring in the different edaphic environments studied (Nemergut et al. 2013). Consequently, dispersal does not seem to significantly shape desert microbial communities despite being intuitively prone to homogenization via wind dispersion (Nemergut et al. 2013).** This was further confirmed by variation partitioning and co-occurrence analyses, which did not detect any stochastic assembly signatures (Dumbrell et al. 2010). Although neutral processes can result in similar patterns of segregation, these patterns are mainly attributed to deterministic environmental filtering (Horner-Devine et al. 2007; Ulrich and Gotelli 2007).

While similar studies have reported high unexplained variation (68-97%) in bacterial assemblages, at a global or local desert scale (Caruso et al. 2011; Ronca et al. 2015; Stomeo et al. 2013), our results demonstrate that more than 50% of variation in community structure across three Domains (Bacteria, Fungi and Archaea) is due to deterministic processes. The proportion of unexplained variation could be due to stochastic influences (e.g. drift or speciation (Caruso et al. 2011)), unmeasured soil physicochemical properties (e.g. metal ion concentration (Gombeer et al. 2015)) or interactions between species (e.g. competition (Caruso et al. 2011; Stomeo et al. 2013)). Interestingly, our results suggest that deterministic assembly mechanisms are the main determinant of edaphic fungal community assembly, in contrast to our original hypothesis based on previous research in this field (Beck et al. 2015; Powell and Bennett 2016).

Conclusions

In this study, we investigated the mechanisms underlying bacterial, archaeal and fungal community assembly in central Namib Desert soils. **Based on previous findings, we expected deterministic processes to be the main drivers of bacterial and archaeal community assembly, and fungal communities to be shaped by stochastic processes (Dumbrell et al. 2010; Powell and Bennett 2016).** Our results, however, show deterministic processes (habitat-filtration/niche-partitioning) to be the predominant drivers of each **Domain** (Nemergut et al. 2013). Interestingly, we observed that contrasting edaphic drivers were found to influence the assembly of microbial community members depending on their soil habitat of origin. This strongly suggests that each Domain should be included in microbial ecology studies to properly evaluate how environmental variables shape microbial communities (Makhalanyane et al. 2015). Furthermore, phylogenetic analysis of the microbial communities through the use of pyrosequencing should be included in future studies to identify which taxa are present**/selected** (van Dorst et al. 2014). This will not only reveal potential functional characteristics of the community, such as ammonia oxidation of AOA and AOB, but also reveal the drivers of individual phyla of the Domains studied here (Fierer et al. 2012; Powell et al. 2015).

Acknowledgements

The authors would like to acknowledge the South African National Research Foundation (grant number N00113-95565) and the University of Pretoria (UP) for supporting this research. We also thank Mr. Charl Herzog of the Department of Plant Production and Soil Science (UP) for providing facilities and assistance with the soil chemistry analysis, as well as the Sequencing and Bioinformatics Unit (UP) for the TRFLP analysis. We also thank MW Van Goethem and AJ van der Walt for helpful comments and insightful criticism regarding the manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest.

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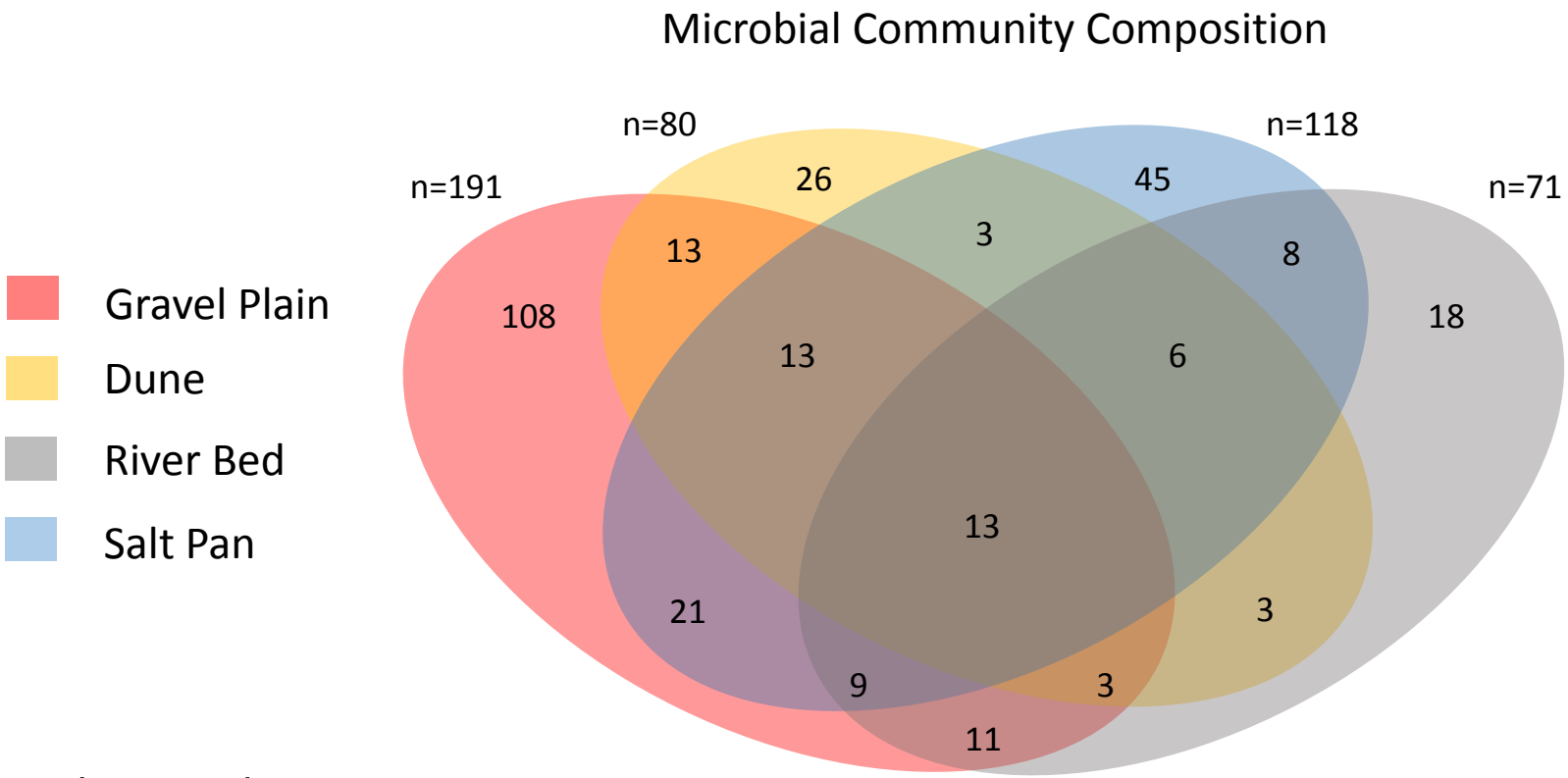
Supplementary material

Supplementary figure 1. Photographs of the nine soil niches sampled; (a) dune top, (b) dune slope, (c) interdune, (d) the wet and dry portion of Hosabes salt pan, (e) hyperarid gravel plain, (f) arid gravel plain, (g) river bed dry and (h) river bed wet soils. Elevation of each site is indicated on the photograph. Photographs a-f provided by Dr. Jean-Baptiste Ramond, photographs g and h provided by Dr. Eoin Gunnigle



Supplementary Figure 2. Venn diagrams illustrating the distribution of OTUs between the four soil types used in this study.