Namib Desert soil microbial community diversity, assembly and function along a natural xeric gradient

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Abstract

The hyperarid Namib Desert is a coastal desert in southwestern Africa and one of the oldest and driest deserts on the planet. It is characterized by a west/east increasing precipitation gradient and by regular coastal fog events (extending up to 75km inland) that can also provide soil moisture. In this study, we evaluated the role of this natural aridity and xeric gradient on edaphic microbial community structure and function in the Namib Desert. A total of 80 individual soil samples were collected at 10 km intervals along a 190 km transect from the fog-dominated western coastal region to the eastern desert boundary. Seventeen physicochemical parameters were measured for each soil sample. Soil parameters reflected the three *a priori* defined climatic/xeric zones along the transect ('Fog', 'Low Rain', and 'High Rain'). Microbial community structures were characterized by T-RFLP fingerprinting and shotgun metaviromics and their functional capacities were determined by extracellular enzyme activity assays. Both microbial community structures and activities differed significantly between the three xeric zones. The deep sequencing of surface soil metavirome libraries also showed shifts in viral composition along the xeric transect. While bacterial community assembly was influenced by soil chemistry and stochasticity along the transect, variations in community 'function' were apparently tuned by xeric stress.

Key words: Aridity gradient / xeric stress / Edaphic desert microbial communities / extracellular enzyme activities / dryland

1. Introduction

Deserts cover more than one-third of the Earth's total land surface, representing the largest terrestrial ecosystem [1]. Worldwide, 5.2 billion hectares of desert lands are used for agriculture, of which an estimated 69% are either degraded or undergoing desertification as a consequence of climatic variation and intensive human activity [2]. Because desert environments contain a limited range of higher plants and animals, soil microbial communities are considered to be the most productive components of these ecosystems as well as the dominant drivers of biogeochemical cycling [3,4].

Compared to more productive edaphic ecosystems, desert soil microbial communities display a generally lower diversity [5-7], which may limit their resistance and resilience to environmental changes [8]. As such, deserts systems may be particularly vulnerable to disturbances such as those linked to global climate change [9]. Global change effects are predicted to induce significant variability in annual precipitation levels in hot deserts, both in time and intensity [10]. Such changes will substantially impact the structures and functions of indigenous microbial communities, as water availability is thought to be the main factor limiting biological processes in arid ecosystems. This observation has led to the theoretical 'microbial-centric' TTRP (trigger-transfer-reserve-pulse) framework [11], where precipitation events act as a trigger to transfer nutrients to soil microbial communities (the reserve) and lead to pulses in biogeochemical activities (e.g., C/N dynamics [11]).

The Namib Desert of southwestern Africa is among the oldest and driest deserts on the planet and its central section has sustained hyperarid conditions for at least the last 5 million years [12]. Rainfall in the Namib Desert is spatially and temporally highly variable, usually of low intensity, but increasing gradually from the coast inland (mean values of 15 to 185 mm per annum for the western and eastern desert margins, respectively; Figure 1 [13, 14]). Due to the cold Benguela Atlantic current, the coast of the Namib Desert is also influenced by regular fog events that can reach as far as 75 km inland and provide up to 183 mm (mean annual) moisture (Figure 1; [13, 14]). This climatic specificity has led to a high level of faunal

and floral endemism in the Namib Desert, including fog-harvesting beetles (*Onymacris, Stenocara* and *Physasterna* spp) [15, 16] and dune grasses (*S. sabulicola*) [17].

The contribution of these two water sources (i.e. rainfall and fog) has led to a well-defined gradient of xeric stress across the Namib Desert (Figure 1) [13, 14]. Moisture source has previously been shown to influence Namib Desert hypolithic microbial community structures, assembly and colonization [18-20], but studies on the effect of water/moisture source on Namib Desert edaphic community diversity and function are limited. A preliminary transect survey across the Namib Desert edaphic has indicated that bacterial community structures are influenced by water source (i.e. fog vs rain; [18]), and a more recent microcosm experiment has established that water regime history is a critical factor in driving bacterial and fungal community structures as well as their adaptation to water stresses [21].

In this study, we established a high resolution 190 km west–east transect across the Namib Desert. Twenty sampling sites were established at 10 km intervals. Based on a large body of long-term climatic data [13,14,22,23], we defined three distinct 'xeric zones': a fog-dominated coastal zone (the 'fog zone'; sites 1 to 6), an intermediate 'low rainfall' zone (the 'Low Rain' zone; sites 7 to 14), and an inland region of higher rainfall (the 'High Rain' zone; sites 15 to 20) (Figure 1). Our working hypothesis is that climate and soil parameters across the xeric gradient should correlate with Namib Desert edaphic microbial community structures, as assessed by T-RFLP fingerprinting [24] and shotgun metaviromics [25]. Similarly, gross microbial functional capacities, as measured by extracellular enzymatic assays [21], were also expected to respond quantitatively to water availability from the coast to the inland desert margin.

2. Materials and methods

2.1. Sampling sites, soil sampling strategy and storage

Twenty sites were sampled at 10 km spacing across a west-east transect in the central Namib Desert on the 22nd and 23rd of April 2013, just before the beginning of the rain season. The transect spanned the three xeric zones defined by long-term climate data (Figure 1; [13, 14, 22]: the 'Fog' zone (F; sites 1 to 6; 15-40 mm precipitation per annum), the 'Low Rain' zone (LR; sites 7 to 14; 55-100 mm precipitation per annum), and the 'High Rain' zone (HR; sites 15 to 20; 101-185 mm precipitation per annum). Recent meteorological data obtained from two weather stations of the SASSCAL network (http://www.sasscalweathernet.org/) located in the Fog (Kleinberg station) and the High Rain (Ganab station) zones of the transect and operational in 2013, showed that April 2013 was dry (1.4 mm and 0.2 mm precipitation, respectively). Furthermore, both stations underwent a precipitation event on the 30th of March 2013 (i.e. approximately three weeks before our sampling expedition took place) of 16.2 mm and 15.7 mm, respectively. From April 2012 until April 2013, both stations presented similar annual averaged temperatures (34.2°C [± 3.9] and 34.6°C [± 3.5], respectively) and total annual precipitation (24.8 mm and 28.6 mm, respectively). Unfortunately, the Vogelfederberg weather station which is located in the Low Rain zone only became operational in 2014. Nevertheless, overall, these data indicate that the general climatic conditions were likely similar for the 20 sampling sites that were sampled along the transect.

At each site, four true replicate soil samples were collected 100 m apart, resulting in a total of 80 individual samples. Vegetation and rocks larger than 1 cm were avoided during collection, as well as disturbed areas such as footprints. Surface soils (0 to 5 cm) were aseptically collected from within a 1 m^2 quadrat into separate sterile Whirl-Pak® plastic bags (Nasco, Fort Atkinson, U.S.A.). Soil samples were stored at 4°C for soil physicochemical analyses, at −80°C for molecular analysis and at −20°C for enzymatic activity assays.

2.2. Soil physicochemical analyses

Seventeen physicochemical properties were measured for each of the 80 soil samples (Supplementary Table S1). Soils were 2mm-sieved and dried at 60°C overnight prior to analysis. Soil texture (i.e., Very Coarse Sand [VCS], Coarse Sand [CS], Medium Sand [MS], Fine Sand [FS], Very Fine Sand [VFS], silt and clay contents) was determined as described by [26] and [27]. Soil pH was determined in a soil slurry at a 1:2.5 soil to deionized water ratio (pH meter Crison Basic +20, Barcelona, Spain). Total soil carbon content was determined using the Walkley–Black acid digestion method [28] and soil organic matter content using the weight loss-on-ignition method (360°C for 2 h; with a 2 h/150°C pre-treatment to remove the soils gypsum crystallized water; [29]). Soil ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were determined using the steam distillation and titration method [30] and soil phosphorus (P) was estimated using the Bray-1 method [31]. Cation exchange capacity (CEC) was determined by ammonium acetate extraction of exchangeable and water-soluble cations [32]. Soil calcium (Ca^+), potassium (K^+), magnesium (Mg^+), sodium (Na^+), and sulfur (S) were extracted with ammonium acetate and the concentrations measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) (SPECTRO Genesis, Ametek, Kleve, Germany) [32].

2.3. Enzymatic assays

The extracellular activity of five enzymes was assessed with chromogenic substrate analogues as described in [21]: β-glucosidase (BG), β-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP), alkaline phosphatase (AP) and phenol oxidase (PO). These enzymes were chosen based on their metabolic functions related to major biogeochemical cycles: carbon-acquiring enzyme (BG), Nitrogen-acquiring enzyme (NAG and LAP), Phosphorous-acquiring enzyme (AP) and lignin-degrading enzyme (PO) [33]. Assays were performed by combining 3 g of soil and 100 mL 50 mM Tris-HCl buffer. Under constant agitation, 200 µL of this soil-buffer slurry was transferred to a 96-well plate. Four replicate wells were used for each sample and controls for both substrate analogue and soil background absorbance were

prepared. Plates were incubated at 43°C (the average daytime soil temperature of the sampling site on collection days) in the dark under constant agitation. After 4h, 10 µl of 0.5 M NaOH was added to each well to terminate the enzymatic activity and the enzymatically induced absorbance changes were measured using a Multiskan™ GO Microplate spectrophotometer (Thermo Scientific, Waltham, U.S.A.). The fluorescein diacetate (FDA) hydrolysis assay, used as a proxy of total microbial activity (e.g., [34]) was performed as previously described [35]. Briefly, 0.5 g of soil was combined with 12.5 mL of 1 × PBS buffer (pH 7.4) and 0.25 mL 4.9 mM FDA dissolved in acetone, and incubated at 43°C for 2 h under constant agitation. After incubation, FDA hydrolysis was halted by adding 40 µl of acetone to 1 ml of soil slurry. Samples were then centrifuged at 8800g for 5 min, and fluorescence (490 nm) was measured with a portable fluorometer (Quantifluor™, Promega, Madison, USA).

2.4. Bacterial community structure analysis

2.4.1. Metagenomic DNA Extraction, 16S rRNA gene PCR amplification and purification

Metagenomic DNA (mDNA) was extracted from 0.5 g soil using the PowerSoil® DNA Isolation Kit (MO BIO, Carlsbad, USA), with minor modifications. Soils from the coastal/fog sites (i.e. sites 1 to 6) were pretreated due to their high salt concentrations and low biomass (Supplementary Table S1) [36]. The pretreatment included three washes with TE buffer (10 mM Tris-EDTA, pH 5.0) centrifuged for 10 min at 7200g prior to mDNA extraction [37]. Six parallel mDNA extractions were performed for the TE buffer washed soils using the MoBio PowerSoil kit (MO BIO, Carlsbad, USA) with a modified elution step: the eluate from the first spin column was used as the eluent for the next spin column as previously described [7]. The extracted DNA was stored at -80°C.

PCR amplification targeting the bacterial 16S rRNA gene was performed using a T100 Thermo Cycler (Bio-Rad, Hercules, U.S.A.). A standard 50 µL reaction volume was used: 0.75% formamide, 0.1 mg/mL bovine serum albumin (BSA), 1 X DreamTaq™ buffer (Thermo Scientific, Waltham, U.S.A.), 0.2 mM of each

dNTP, 0.5 µM of fluorescent-labeled forward primer 341F [38] (5'-CCTACGGGAGGCAGCAG-3'), 0.5 µM of reverse primer 908R [39] (5'-CCGTCAATTCCTTTRAG-TTT-3'), 0.005 U/µL DreamTaq™ DNA polymerase (Thermo Scientific, Waltham, USA) and 1 µL of metagenomic DNA as template. The cycling conditions consisted of an initial denaturation step of 5 min at 95°C; 20 amplification cycles of 95°C for 30s, 55°C for 30s, and 72°C for 90s; and a final extension step at 72°C for 10 min. PCR products were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Duren, Germany) in accordance with the manufacturer's protocol.

2.4.2. Terminal restriction fragment length polymorphism (T-RFLP)

Purified PCR amplicons (400 ng) were digested using the FastDigest® *Msp*I restriction endonuclease (restriction site C^CGG) (Thermo Scientific, Waltham, U.S.A.) for 15 min at 37°C. Digested products were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey-Nagel, Duren, Germany) prior to capillary electrophoresis at the DNA Sequencing Facility of the University of Pretoria (South Africa) using an ABI 3500 XL Genetic Analyzer (Applied Biosystems, Foster City, U.S.A.).

2.5. Viral DNA extraction, amplification and sequencing

The metaviromic DNA of soil samples from 4 sites (4, 7, 10 and 13; Figure 1) was extracted according to [40], with slight modifications. Five grams of soil (pooled from the 4 true replicates collected at each site) were added to 15 ml of 1% potassium citrate buffer and vortexed at full speed for 15 seconds. The mixture was incubated on ice for 25 min, followed by three cycles (30% amplitude for 59 sec) of sonication with an ultrasonic processor using a 1/16" probe tip. Samples were centrifuged at 3000g at 4°C for 30 minutes. The supernatant was decanted, transferred to a new tube and passed through a 0.20 µm cellulose acetate sterile syringe filter (GVS). Viruses and virus-like particles were concentrated by adding 25% PEG8000 (in 1M NaCl) to the filtrate to a final concentration of 10% (w/v) and incubated overnight at 4°C. Concentrates

were centrifuged for 30 minutes at 32000g at 4°C. The supernatant was decanted and the viral pellet re-suspended in 300 µl phage buffer (10mM Tris-HCl, 10 Mm Mg SO4, 150 mM NaCl, pH 7.5). Viral concentrates were treated with DNase I (Thermo Scientific, cat#EN0523) and RNase A (Thermo Scientific, #EN0531) according to the manufacturer's instructions. Viral DNA was purified using the Quick-gDNA MiniPrep kit (Zymo Research, cat# D3025) according to the manufacturer's instructions and randomly amplified using the REPLI-g Midi kit (Qiagen, cat# 150043) according to the manufacturer's instructions. Amplified DNA was precipitated with isopropanol, washed with 70% ethanol and re-suspended in 25µl milli-Q water.

The amplified metaviromes were checked for bacterial contamination by assessing the presence of the 16S rRNA gene by PCR amplification as described above. Library building for sequencing was done using the Ion Xpress™ Plus and Ion Plus Library Preparation for the AB Library Builder™ System (Publication Number MAN0006946). Template amplification was done using the Ion OneTouch™ 2 System (OT2) Ion PI™ Hi-Q™ OT2 200 Kit (Number MAN0010857). The metavirome libraries were multiplexed and sequenced using the Ion PI™ Hi-Q™ Sequencing 200 Kit (Number MAN0010947) using the Ion PI™ Chip Kit v3. Sequencing was performed on the Ion Proton platform, located at the Central Analytical Facilities, Stellenbosch University, South Africa.

2.6. Data Analyses

Physicochemical data were normalized in Primer6 and visualized using a correlation-based principal component analysis (PCA) to determine the dominant environmental gradients of the transect samples (Primer-E Ltd, Devon, UK) [41]. Functional data were Hellinger-transformed [42], and combined with the environmental parameters measured, were visualized in a redundancy analysis (RDA) plot with Bray-Curtis dissimilarity matrices [43] in Primer6 (Primer-E Ltd, Devon, UK).

T-RFLP profiles were analyzed using Gene Mapper® software (Applied Biosystems, Foster City, USA). Terminal restriction fragments (T-RFs) smaller than 50 bp and greater than 600 bp were eliminated, and a baseline threshold of 20 fluorescence units was used to delineate background noise. Peaks were then binned into Operational Taxonomic Units (OTUs) with custom scripts (standard deviation 1.5) using R [44, 45]. OTU relative abundances were Hellinger-transformed [42] and were also combined with the edaphic parameters measured in a RDA. Variation partitioning and co-occurrence null model analyses were performed as previously described [24].

(PERM)ANOVA ([Permutational] analysis of variance) was used to identify significant differences between groups of samples using R. Using the PAST v3.14 software package, we tested for relationships between the 'distance to coast' (km) and the different soil enzyme activities. The latter were ln(x+0.5) transformed to achieve near normal distribution. Ordinary Least Square (OLS) was first used to evaluate linear relationships between 'distance to coast' (km) and the soil enzymatic activities. If unsuccessful, we tested for nonlinear relationships by using polynomial regression. A partial Mantel test was performed to evaluate correlations between the functional (enzymatic) and diversity (T-RFLP) matrixes using R (999 permutations).

2.7. Metavirome sequence analyses

Metavirome sequence reads were curated for quality and adapter trimmed using CLC Genomics version 6.0.1 (CLC, Denmark), using the default parameters. *De novo* assembly for each read dataset was performed with the CLC Genomics assembler suite using the default parameters. Contigs were uploaded to and are available for analysis from the following online pipeline: the MetaVir version 2 server ([46]; http://metavir-meb.univ-bpclermont.fr/). The four metavirome read datasets are also available from the Sequence Read Archive of NCBI under the accession no. ERX1230691 to ERX1230694 [25]. Taxonomic composition by MetaVir was computed from a BLASTp comparison with the Refseq complete viral

238 genomes protein sequences database from NCBI (release of 2015-01-05) with an E-value threshold of 10^{-5}. Unique and shared virus hits were determined by recording the occurrence of all virus isolate hits (contig best blast hit number, E-value threshold 10^{-5}, MetaVir) in each soil sample dataset, and visualized using the Venn diagram online tool, available from the Bioinformatics and Evolutionary Genomics group website (http://bioinformatics.psb.be/webtools/Venn/). The term "viral operational taxonomic unit" ("vOTU") is used here to describe contigs with a taxonomic assignment based on the best BLAST hit (BLASTp query against the RefSeq database, 10^{-5} threshold on the E-value).

3. Results and Discussion

Aridity in drylands has been shown to influence the structure and function of soil microbial communities although results are often contradictory. At the global scale (80 sites located on 5 continents), bacterial and fungal diversities and abundances increased with decreasing aridity [47] while, at the local scale (within the country of Israel), soil bacterial abundances also decreased with aridity but diversity remained constant [48]. Furthermore, while soil pH is strongly affected by aridity [47], microbial extracellular enzyme distribution has been found to generally be influenced by soil pH [49, 50] and not by mean annual precipitations [50]. In the Namib Desert, however, microbial extracellular enzyme activities were found linked to water regime histories (riverbed vs gravel plain) and not by pH [21].

These contradictions suggest that our knowledge of arid land microbial ecology must be improved, most particularly as (i) the vast majority of dryland ecosystem processes are microbially-mediated [3, 4] and (ii) predictive modeling shows that global surface area of arid land will increase [51]. This experiment was therefore designed to study the structure and function of edaphic microbial communities across a naturally occurring xeric stress gradient (Figure 1).

3.1. Soil physico-chemical properties

A principal component analysis (PCA) plot based on 17 soil physicochemical parameters (Figure 2; Supplementary Table 1) showed that the soils from the three *a priori* defined xeric zones ('Fog', 'Low Rain', and 'High Rain' zones) were clearly separated along PCA axis 1 (which explains 30.6% of the sample variation; Figure 2a). The clusters were strongly correlated with soil pH, 'coarse sand' content and Ca^{+}, S, Na^{+} and NO_3^{-} concentrations (Figures 2b, 2c). PERMANOVA confirmed that the soil physicochemistries of each zone were significantly different (PERMANOVA p = 0.001; Table 1), supporting a previous study which observed that within the Namib Desert gravel plains, multiple lithologies (e.g., schist, granite, surficial cover and salt crusts) and geological units (e.g., Kuiseb, Salem, Surficial cover, Saline spring) can be found

[52]. In general, the ionic (Ca^{2+}, K^{+}, S^{2-}, Mg^{2+}, Na^{+} and NO_3^{-}) content of the fog zone soils was higher than in those of the rain zones (Supplementary Tables 1 and 2). We attribute this effect to the coastal transport and deposition of marine aerosols [53, 54] rather than fog input: the low ionic content of fog precipitation suggests that fog events have little impact on the soil chemistry [55, 56]. The 'High Rain zone' soils were characterized by significantly higher soil organic matter than all other transect soils (ANOVA $p < 0.05$; Supplementary Tables 1 and 2). We attribute this to the generally higher plant productivity in this region [57], as compared to those of the Fog and Low Rain zones.

3.2. Namib Desert microbial community

Each xeric zone showed significantly different microbial community structures and bacterial community functional capacities (PERMANOVA, $p < 0.05$; Table 1). In particular, the community structures and activities of the fog zone soils clearly separated from those of the rain zone soils, essentially due to their higher salt, principally cation, content (Figure 3). These parameters are well-known environmental filters for microbial communities [58].

3.2.1. Bacterial community structure and assembly

The bacterial communities in the low and high rain zone samples showed higher α- and lower β-diversities than those of the fog zone (Table 2). We used variation partitioning and co-occurrence null model analyses to evaluate the assembly of the bacterial communities in the different xeric zones (Table 2; [18, 24]). The combination of spatial (xeric zone) and environmental (soil chemistry) parameters explained 37.5% of the variation in the assembly of bacterial communities along the transect. This result strongly suggests that stochasticity plays a major role in Namib Desert bacterial community assembly [59]. Furthermore, only 7% (0.026/0.375; Table 2) of the variation of the bacterial community assembly along the transect was attributed to the xeric zonation, while soil physicochemestries explained 53% (0.2/0.375; Table 2),

294 indicating that the historical nature and intensity of their precipitation (fog, light rain or high rain) is not a
295 critical factor. This further confirmed that local edaphic physicochemical environments are significant in
296 shaping Namib Desert bacterial communities [52] and that climate (e.g., fog) has little impact in
297 pedogenesis in the central Namib Desert [14]. However, null model analysis indicated that the co-
298 occurrence of OTUs was non-random (Table 3), suggesting that a combination of deterministic and
299 stochastic processes [60, 61] are involved in microbial community assembly along the Namib Desert
300 longitudinal transect. The high and positive standardized effect size (SES, Table 3) also suggested that
301 biological interactions play a role [62] in the assembly of Namib Desert edaphic communities. This would
302 appear to contradict the results obtain in our recent study [24] which showed that Namib Desert edaphic
303 communities assembled primarily by deterministic processes (e.g., niche speciation). However, in that
304 study, communities from highly contrasted soil biotopes (dunes, gravel plains, riverbeds, and salt pans)
305 were included while, here, we focused on a single more homogeneous biotope: the Namib Desert gravel
306 plain soils. We conclude that, depending on the scale of observation, community dynamics can vary (e.g.,
307 metacommunity vs local community; [63]).
308
309 3.2.2. Namib Desert soil extracellular enzymatic activities
310 We measured the extracellular activities of five enzymes commonly used as proxies for soil microbial
311 nutrient demand [21, 33, 50]. Extracellular enzyme activities were calculated as absorbance change 'per
312 g dry soil' (gDS).h^{-1}, which is accepted as an ecosystem-level measure of microbial activity and allows for
313 direct comparison of activities between samples [64, 65].
314 Significant relationships between 'distance-to-coast' of the sampling sites and the activities of five of the
315 six enzymes tested were detected (Figure 4); i.e., fluorescein diacetate (FDA) hydrolysis, β-glucosidase
316 (BG), alkaline phosphatase (AP); leucine aminopeptidase (LAP) and phenol oxidase (PO) activities. Because
317 of the strong and significantly positive linear relationships between the distance from the coast and the

annual rain precipitation ($r^2 = 0.94$, $p < 0.001$; data not shown), the role of long-term climatic parameters could not be excluded as a factor in explaining the activities of the Namib Desert edaphic communities. Extracellular enzyme activities in the high rain zone were generally higher than in the low rain and fog zones (Figure 4; Supplementary Table 3). This was expected as soil moisture is known to directly influence the levels of extracellular enzyme activities in drylands systems [66] and their activities are strongly simulated by the abundance of water availability following rainfall events [67].

A significant relationship between community structure and function was found (Mantel test; $r = 0.2$; $p < 0.01$). This confirmed that microbial community composition is critical for certain processes to be performed in desert environments [7,20].

3.3. Viral community composition

Multiplexed sequencing of the viral communities from site 4 of the Fog zone and sites 7, 10 and 13 of the 'Low rain' zone (Figures 1 and 5) produced 93,519,306 reads (~13.4 Gb), yielding approximately 22 million reads per metavirome. The mean read length was 142.5 bp and the mean GC content ranged from 54 to 62%. Bacterial contamination in the metaviromes was negligible, as no amplification of rDNA was observed and no rDNA sequences were identified by the MG-RAST pipeline. Across all soil samples, the ratio of taxonomically assigned to unassigned sequences ranged from 9.2 to 18.9%, indicating a highly uncharacterized pool of viral diversity and supporting the idea that viral populations are still poorly characterized in arid environments [68,69]. Rarefaction curves for all metaviromes remained linear (Supplementary Figure S1), indicating that the datasets substantially underrepresented the complete viral diversity within each sample. In site 4 (fog zone) the dominant hits were assigned to *Mycobacterium* phage Adler (6.5%) and *Rhizobium* phage 16-3 (4.4%), both unclassified members of the *Siphoviridae* family of tailed phages (Order: *Caudovirales*). Members of the nucleocytoplasmic large DNA virus (NCLDV) families *Mimiviridae* and *Phycodnaviridae* were also common in the site 4 sample (Figure 5a). Single-stranded DNA

342 (ssDNA) viruses were only detected in the Low Rain sites (2.4% in site 7, 0.7% in site 10 and 6.8% in site
343 13), despite the use of a DNA amplification method biased towards the detection of circular ssDNA viruses
344 (Figure 5a). This is in stark contrast with salt pan sites located in the 'Fog' and 'Low Rain' zones which
345 contained a high diversity of ssDNA viruses [70], leading to the hypothesis that (the hosts of) these viruses
346 are not well adapted to edaphic environments.
347 Sites 10 (n = 548 vOTUs) and 13 (n = 366 vOTUs) of the Low Rain zone presented richer viral communities
348 when compared to those of the Fog zone (site 4: n = 43 vOTUs; site 7: n = 75 vOTUs; Figure 5b). This trend
349 being also observed for the bacterial communities, which showed higher α-diversities in the rain zones
350 (Table 2), it supports the conclusion that edaphic virus communities reflect the microbial host diversity
351 [69]. Of the 1032 individual vOTU detected, only 3 (0.3%) were observed in all 4 samples (Figure 5b),
352 namely, *Streptomyces* phage mu1/6, *Yersinia* phage phiR1-37 and *Cellulophaga* phage phi19:1), while 295
353 vOTUs (66.4%) were exclusive to single sampling sites (Figure 5a). It is noteworthy that the 3 cosmopolitan
354 vOTUs were all assigned to viruses infecting bacterial phyla which are known to be dominant in desert
355 soils; i.e., Actinobacteria, Proteobacteria and Bacteroidetes [4]. However, while *Streptomyces* spp. are
356 common in Namib Desert soils [4] and *Yersina* phages have already been detected in desert soils [71], the
357 detection of the marine *Cellulophaga* phage phi19:1 [72] throughout the transect was unexpected.
358 Marine-phage sequences have recently been detected in a ~100km inland Namib metaviromic study [73],
359 and our result, therefore, tend to confirm their hypothesis that marine fog and wind play a role in the
360 dispersal of (marine) phages into Namib Desert soils. Assuming that viral community composition mirrors
361 the host community structure [69, 74], the observation of marine phage signals in inland desert soils is
362 also in line with our finding that both stochasticity (principally via dispersal) and determinism (i.e., niche
363 partitioning) (Table 2) are involved in the assembly of Namib Desert gravel plain microbial communities.
364

365 **4. Conclusions**

As initially hypothesized, Namib Desert microbial community structures were significantly different in the three *a priori* defined xeric zones along the longitudinal desert transect (Figure 3). However, while soil physicochemistry was identified as a statistically significant factor in microbial community assembly, water regime history (i.e., the xeric zonation) was not determinant (Table 2). This strongly suggests that adaptation to the immediate edaphic environment is a stronger environmental filter for soil communities than long term climatic patterns in desert ecosystems. We argue that microbial communities in desert soils experience (hyper)arid conditions for much of any given time period, and that, while differences in precipitation in the xeric zones are significantly different in terms of volumetric loads, their biological impact was not (Table 2). Furthermore, precipitation events are generally highly localized in desert systems, particularly in the Namib Desert [14]. Contrastingly, microbial community functionality, as indicated by soil extracellular enzyme activities, increased from the coast inland (Figure 4), confirming that long-term precipitation patterns (or different xeric stresses) play a role in the structuring of desert edaphic microbial community functionality [21].

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Figure Legends

Figure 1. Map showing the distribution of sampling sites in the Namib Desert across the longitudinal west/east xeric gradient Map showing the distribution of sampling sites in the Namib Desert across the longitudinal west/east xeric gradient. Adapted from [13, 14, 22]. Image produced using Google Earth, © 2016 DigitalGlobe.

Figure 2. Results of the Principle Component Analyses (PCA) using the 17 Namib Desert soil variable recorded. a. PCA ordination plot. Correlation circles showing the relationships between the environmental variables and the first two PCA axes: the soil particle sizes (**b**) and the chemical descriptors (**b**). The descriptors were separated in two separate correlation circles for clarity. Variables that are correlated with the first two axes of the PCA plot are the most important in explaining the variability in the data set. Vectors indicate the strength (length) and direction (arrow orientation) of the variables in the ordination. Coarse, Med, Fine: Coarse, Medium, Fine sand content, respectively; C: Carbon; CEC: cation exchange capacity; Ca^{+}: Calcium; K^{+}: Potassium, Mg^{+}: Magnesium; Na^{+}: Sodium; NH_4^{+}: Ammonium; NO_3^{-}: Nitrate; OrgMatter: Organic Matter content; Phos: Phosphorus; S: Sulfur). ■ = 'Fog Zone', ■ = 'Light Rain Zone' and ■ = 'High Rain Zone'.

Figure 3. Redundancy analysis (RDA) bi-plots displaying the influence of soil physicochemistries on Namib Desert (a) edaphic bacterial community structures and (b) global soil functional capacities. Only the environmental variables that significantly ($p < 0.05$) explained variability in microbial community structures are fitted to the ordination (arrows). The direction of the arrows indicates the direction of maximum change of that variable, whereas the length of the arrow is proportional to the rate of change. ■ = 'Fog Zone', ■ = 'Light Rain Zone' and ■ = 'High Rain Zone'.

Figure 4. Relationships between the Namib Desert soil enzymatic activities and the distance to the coast. When significant, the linear or nonlinear relationships are indicated on the plot along with the equations and r^2 values. Bootstrapped 95% confidence intervals (1999 replicates) border the OLS linear regression lines. The enzymatic activity used were calculated as 'per g dry soil' (gDS). ■ = 'Fog Zone' activities, ■ = 'Light Rain Zone' activities and ■ = 'High Rain Zone' activities.

Figure 5. Diversity of the Namib Desert soil metaviromes in the four transect soil studied. a. Family level taxonomic compositions computed from a BLAST comparison with NCBI RefSeq complete viral genomes proteins using BLASTp (threshold 10-5 on the e-value). Virus hit numbers were normalized and converted into ratios. The unclassified category includes all dsDNA and ssDNA viruses. **b.** Venn diagram showing the distribution of unique and shared viral OTUs. "n" indicates the total number of vOTUs detected in each site.

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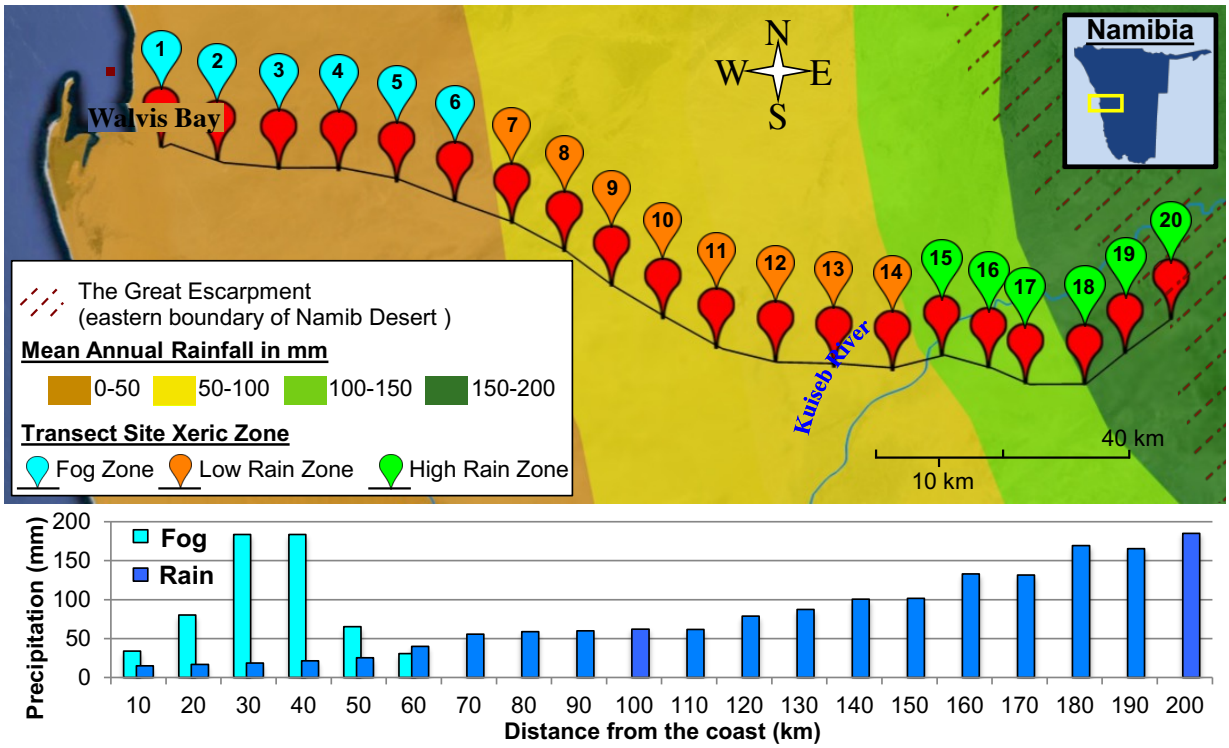
625 **Figure 1**



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628 **Figure 2**
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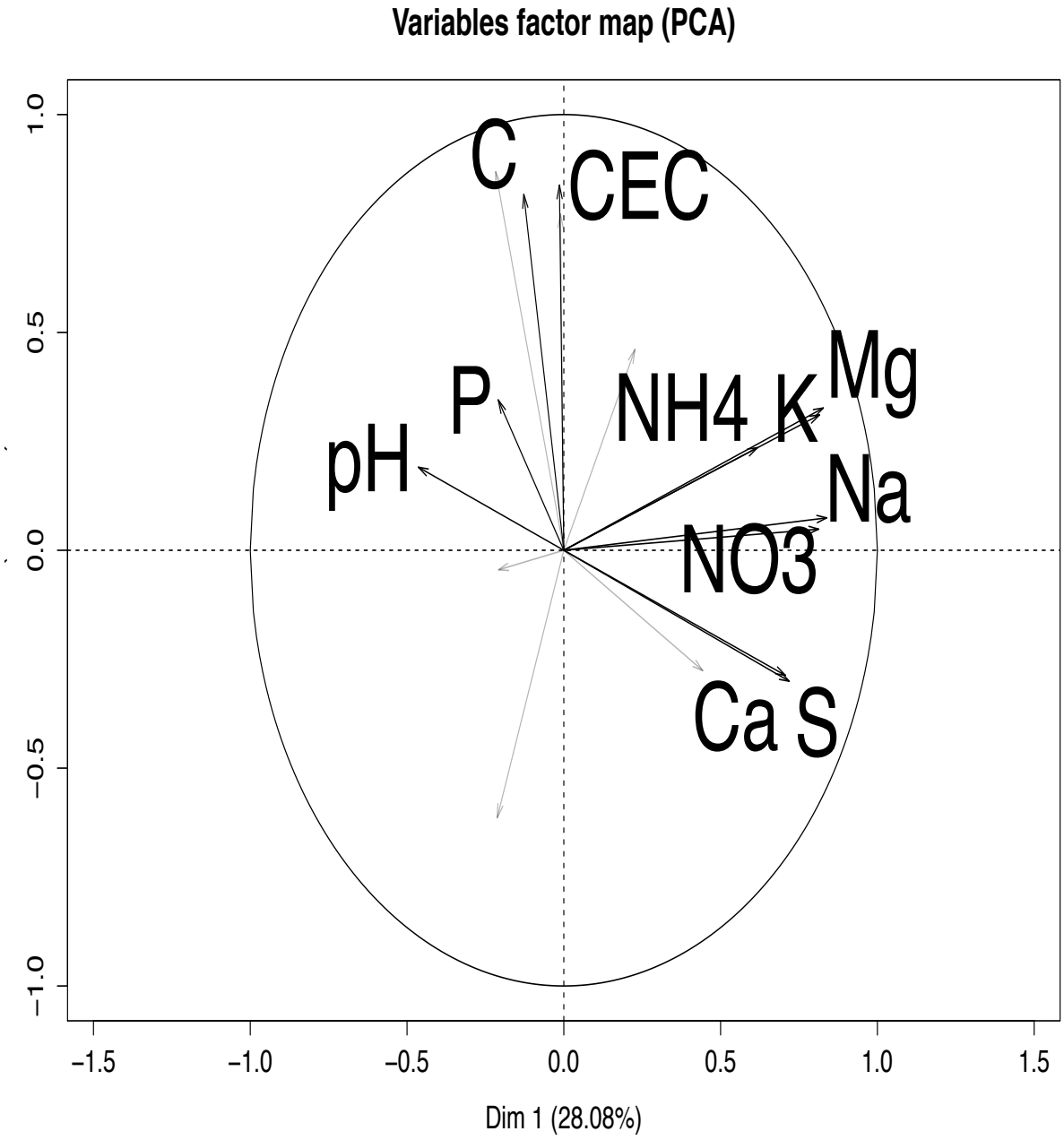

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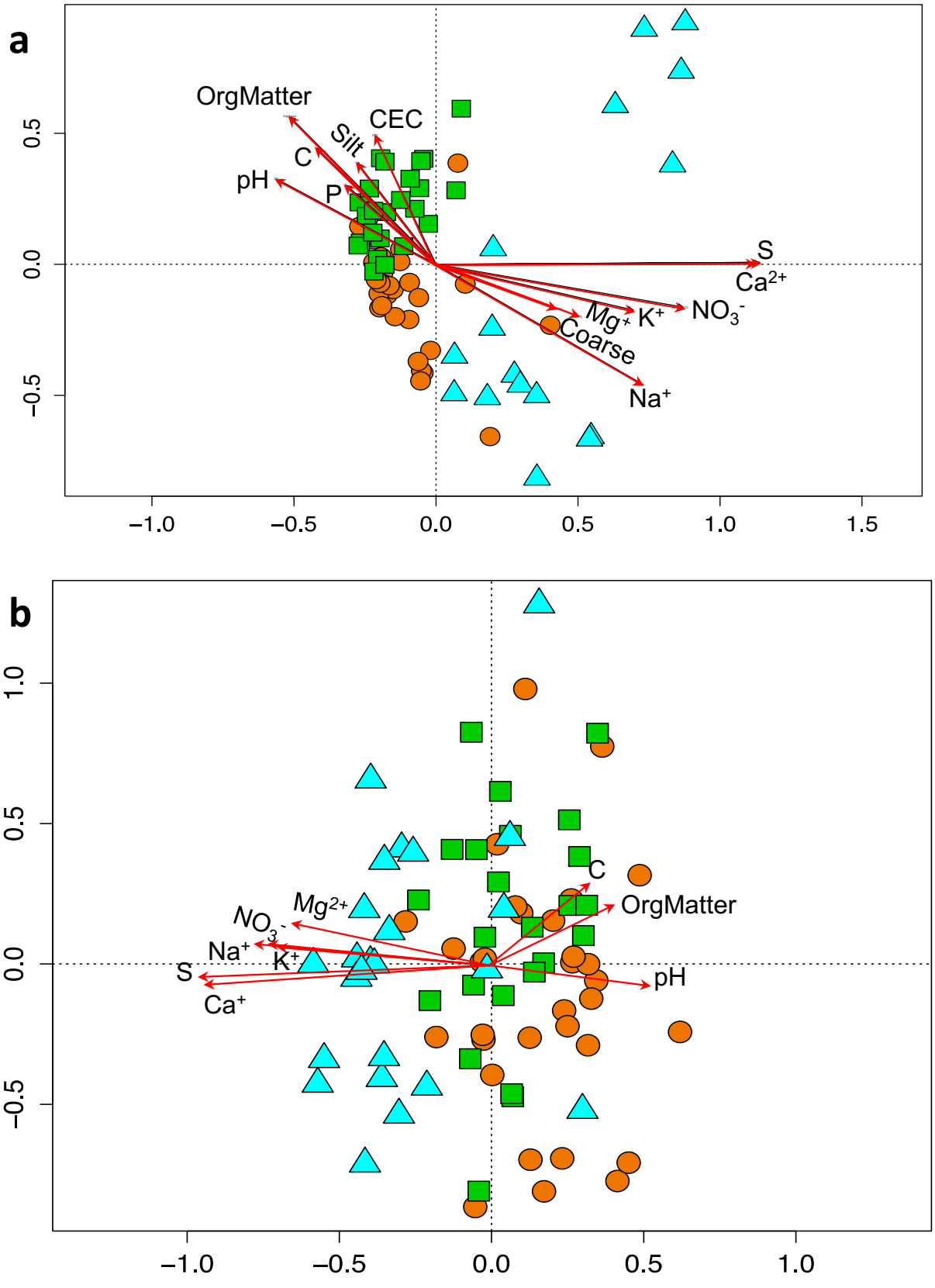

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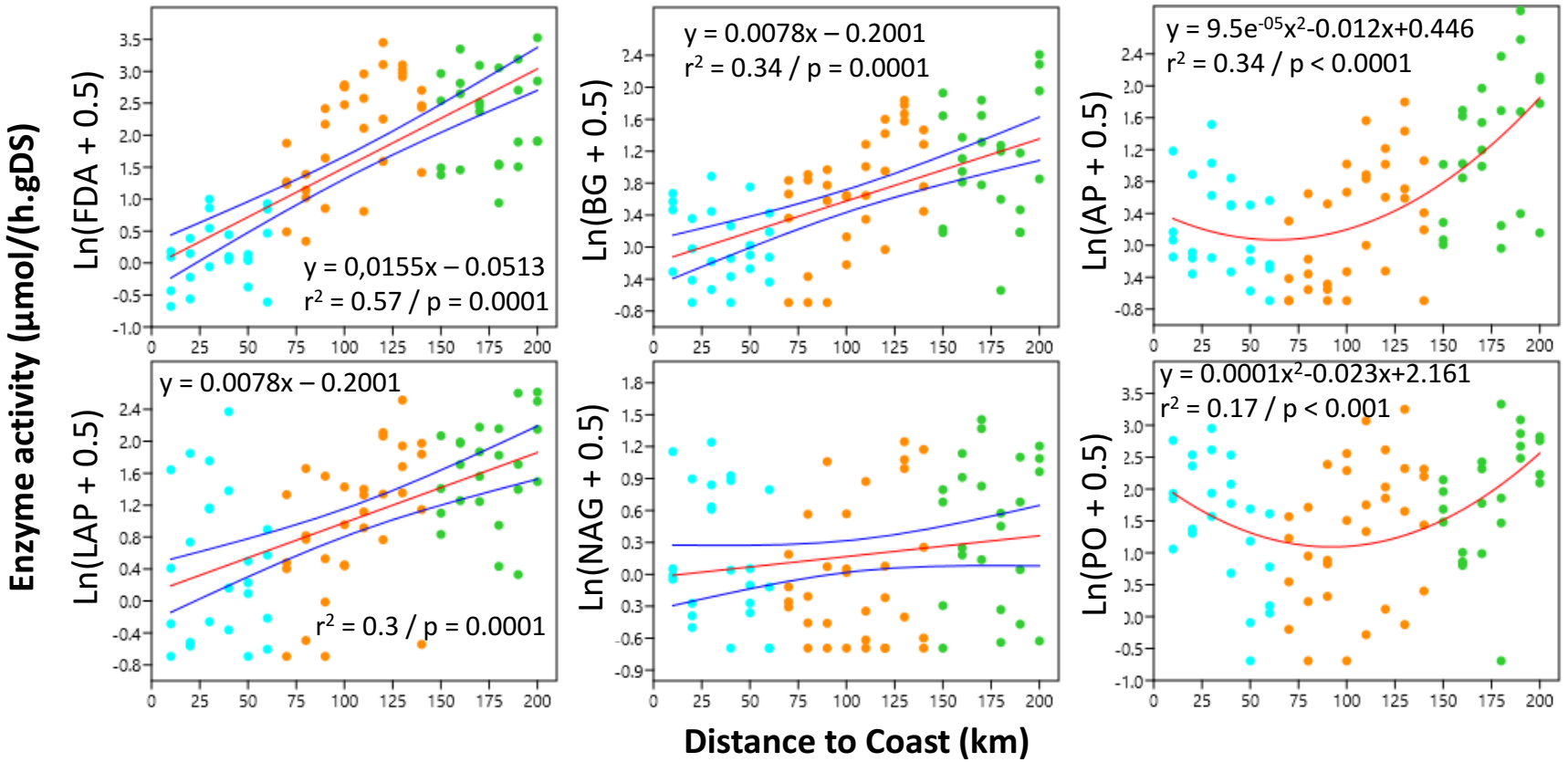

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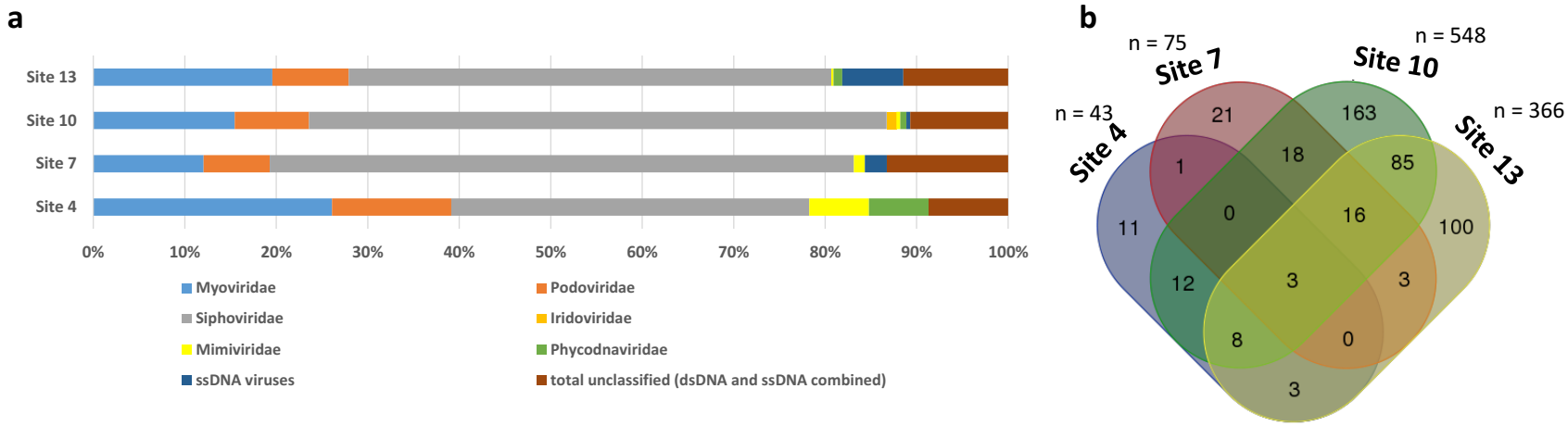

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Supplementary Figure S1. Rarefactions curves of Namib soil metaviromes. Rarefaction curves were generated based on a clustering of the predicted protein genes. Clustering (i.e. grouping) of predicted protein sequences was done through the detection of conserved domain (using the PFAM database) with a similarity threshold of 75%). The curve represents the number of different clusters created (y-axis) from a given number of sequences (x-axis).