Temporal dynamics of microbial transcription in wetted hyperarid desert soils

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Abstract

Rainfall is rare in hyperarid deserts but, when it occurs, it triggers large biological responses which are considered to be essential for the long-term maintenance of biodiversity. In such environments, microbial communities have major roles in nutrient cycling, but their functional responses to short-lived resource opportunities are poorly understood. We used whole community metatranscriptomic data to demonstrate structured and sequential functional responses in desiccated desert soils to a simulated rainfall event over a seven-day time frame. Rapid transcriptional activation

of Actinobacteria, Alpha-proteobacteria and phage transcripts was followed by a marked increase in protist and myxobacterial activity, before returning to the original state. In functional terms, motility systems were activated in the early phases, whereas competition-toxicity systems increased in parallel to the activity from predators and the drying of soils. The dispersal-predation dynamic was identified as a central driver of microbial community responses to watering. Carbon fixation mechanisms that were active under dry condition were rapidly down-regulated in wetted soils, and only reactivated on a return to a near-dry state, suggesting a reciprocal balance between carbon fixation and fixed-carbon acquisition processes. Water addition induced a general reduction in the transcription of stress response genes, most prominently HSP20, indicating that this chaperone is particularly important for life in desiccated ecosystems. Based on these data, we propose a rainfall response model for desert soil microbiomes.

Introduction

Arid lands cover approximately one third of the terrestrial surface (Laity, 2009). Aridity, as a result of very low precipitation and high potential evapotranspiration rates, severely limits biomass production and promotes oligotrophy (Delgado-Baquerizo et al., 2013; Maestre et al., 2015), eventually leading to habitat fragmentation where productivity is concentrated in sheltered "islands" (Pointing and Belnap, 2012; Schlesinger et al., 1995). Microorganisms in open soils outside of these privileged micro-environments are subjected to an intense desiccation stress which limits their biological activity (Lebre et al., 2017). However, desert edaphic microorganisms are regarded as important for the long-term fertility of soils and for

post-rainfall grass germination (Delgado-Baquerizo et al., 2016). The Namib Desert, on the south-western coast of Africa, is the oldest continuously hyperarid desert in the world and provides an excellent model of a stable hyperarid ecosystem (Seely et al., 2008). It is generally accepted that much of the microbiome in such environments exists in a state of dormancy (Bär et al., 2002; Lebre et al., 2017), but responds with dramatic changes in structure and activity when water becomes available (Armstrong et al., 2016; Austin et al., 2004; Collins et al., 2014; Garcia-Pichel and Pringault, 2001; Štoviček et al., 2017). Recent studies have, however, shown that some transcriptional activity is detectable in hyperarid soil microbial communities during prolonged dry periods (Gunnigle et al., 2017; León-Sobrino et al., 2019; Schulze-Makuch et al., 2018). Since arid lands represent a large part of the terrestrial surface of the Earth and are currently expanding through desertification processes (Huang et al., 2016), a deeper understanding of these pulsed phenomena is of global relevance.

Research on desert soil microbial ecology has primarily focused on bacterial communities, suggesting that extreme abiotic pressures, such as high temperature, desiccation stress and UV radiation are dominant drivers of both the diversity and function of bacterial communities (Frossard et al., 2015; Johnson et al., 2017; León-Sobrino et al., 2019; Mandakovic et al., 2018; McHugh et al., 2017; Ronca et al., 2015; Scola et al., 2018; Vikram et al., 2016). By comparison, virus/phage communities in hot desert soils have been relatively under-studied (Trubl et al., 2020; Williamson et al., 2017), despite the fact that they represent one of the most abundant and genetically diverse entities on Earth and are inferred to play central ecological roles in biogeochemical nutrient cycling in diverse environments (Adriaenssens et al., 2017;

Bezuidt et al., 2020; Luo et al., 2020; Zablocki et al., 2018). Furthermore, phages (i.e., viruses that infect bacteria) have been shown to affect community turnover and resource availability in soils and to globally drive microbial evolution via horizontal gene transfer (Rodriguez-Valera et al., 2009; Trubl et al., 2019; Williamson et al., 2017).

In this study, a gravel plain soil plot in the central Namib Desert was subject to an artificial rainfall pulse of 30 L/m², the approximate average annual rain received in this region (25 mm) (Eckardt et al., 2013) and sufficient to stimulate plant germination (Seely et al., 2008). The short-term responses of the soil microbiome were monitored using whole-transcriptome analysis of gene function. Based on this analysis, we propose a structured water response model with differentiated phases and trophic interactions.

Materials and methods

Surface soils were collected from two adjacent 3.5 x 3.5 m plots (approx. 10m separation) in the central Namib Desert gravel plains (23° 33' 18" S, 15° 3' 20" E). The control plot remained dry, while the experimental plot was manually watered (30L/m², simulating a 30 mm rainfall event) at T₀ (26th April 2017 10:00 AM WAT/UTC+1), using a synthetic "Namib Desert rain" solution, prepared from ultrapure DNA-free water supplemented with a defined salt mixture (Frossard et al., 2015). Plots were subdivided into 0.5 x 0.5 m quadrats and randomly sampled, in triplicate, at specified times after water addition (Supplementary Figure S1). 20 g samples of surface (0-5 cm) soil were collected at 10 minutes; 1, 3 and 7 hours; and 1, 3 and 7 days after simulated rainfall, preserved immediately in RNAlater solution (Sigma-Aldrich, St. Louis MO, USA) and subsequently frozen at -20°C prior to RNA extraction. Additional 200 g soil samples

were collected from the same locations into WhirlPak™ bags (Nasco, Fort Atkinson WI, USA) and frozen for subsequent soil chemistry analysis.

The water content of soil samples (sieved to <2 mm particles) was measured gravimetrically for three days after watering. Soil silt, sand, and clay compositions were measured by the hydrometer method (Gee and Bauder, 1986). Particle size distributions were determined by sieve separation (> 1000 µm, > 500, > 250, > 100, > 53 and < 53 µm). pH, electrical conductivity, Na, Cl, K, Ca, Mg, NO₃, NH₄ and P composition were analysed at Bemlab (Pty) Ltd. (Strand, Western Cape, South Africa) using standard protocols. Soil organic carbon percentage was measured using the Walkley-Black test (Walkley, 1935).

RNA was extracted following the protocols described in León-Sobrino *et al.* 2019 (León-Sobrino et al., 2019) from triplicate soil samples. In order to mitigate the effect of chemical variation, the two biological replicates most similar to the average chemical composition of all sampled soils were selected for RNA extraction. Stranded, rRNA-depleted libraries were prepared with the ScriptSeq Complete Gold Kit (Epidemiology) (Illumina, San Diego, USA) and 150 bp paired-end sequences were read on a HiSeq4000 platform (Illumina).

Sequencing outputs were processed using the BBtools suite v. 38.26 (Bushnell et al., 2017) (<https://sourceforge.net/projects/bbmap/>). Read ends below a quality Phred value of 20 were trimmed using *BBDuk*; rRNA and human RNA sequences were identified and removed using SILVA v. 111 (July 2012) (Quast et al., 2012) and 5S RNA (Szymanski et al., 2016) databases and a curated human genome reference assembly hg19 (<https://drive.google.com/file/d/0B3lHR93L14wd0pSSnFULUIhcUk>) (bbmap,

2018) following recommended protocols. Optical duplicates generated by the patterned sequencing flowcell were removed using the *Clumpify* function from the BBtools suite, setting the distance cut-off to 2,500 pixels. Transcript assembly was performed using transAbyss v.2.0.1 (Robertson et al., 2010) for each library. Individual assemblies were merged with the same software (*transabyss-merge* function) to generate a reference metatranscriptome.

Contigs were annotated at the Integrated Microbial Genomes and Microbiomes (IMG/M) server (Huntemann et al., 2015) (<https://img.jgi.doe.gov/>). Predicted protein products from genes were also analysed against the Conserved Domain Database (CDD) using Delta Blast (e-value threshold 10^{-4}) (Boratyn et al., 2012; Marchler-Bauer et al., 2015). Contig taxonomy was determined by a consensus among all individually classified genes, requiring a *quorum* of >50% at each taxonomic level.

Reads were aligned to the reference assembly using BBmap (Bushnell et al., 2017) and reads for annotated regions in each library were counted using FeatureCounts v. 1.6.3 (Liao et al., 2014). The assembled counts matrix was aggregated along functional and/or taxonomic categories as required for each analysis.

Differential transcription along the time series was analysed with the DESeq2 v. 1.14 package in R (Love et al., 2014), comparing data from the control (dry) soil samples with those from the experimental (wetted) samples from the same point in the time-series. Transcripts that significantly diverged in abundance from the control at any given time were considered up-regulated in response to watering (adjusted p-value ≤ 0.05 for the likelihood ratio test). Transcripts per million (TPM) of ribosomal protein genes as a fraction of the total for their respective taxonomic group (Rp:T)

were employed to estimate absolute activity of each group along the time course, following the premise that ribosome densities in a cell relate to metabolic activity and growth rates (Bosdriesz et al., 2015; Bremer and Dennis, 1996).

Viral contigs were identified from assembled transcriptomes using VirSorter v. 1.0.3 (Roux et al., 2015) and the virome database on the iVirus platform hosted by CyVerse (Bolduc et al., 2017). Only contigs >1 kb, and classified as categories 1, 2, 4, and 5 were considered (phages and prophages identified with the “pretty sure” and “quite sure” qualification). To calculate the relative abundances of the different viral contigs in each transcriptome, quality filtered metatranscriptomic reads were mapped back to the viral contigs with Bowtie2 v. 2.2.6, using the default parameters (Langmead and Salzberg, 2012). The output SAM files were converted into BAM files, sorted and indexed, using SAMtools (Li et al., 2009). A python script was used to generate a coverage profile of viral contig abundances across samples.

ORFs were predicted within putative viral contigs using Prodigal (Hyatt et al., 2010). Transcripts per million (TPM) putative viral protein genes were employed to normalize the final ‘viral OTU’ (vOTU) values for each sample. Predicted protein sequences were clustered with proteins from viruses in the NCBI ViralRefSeq-prokaryotes-v85 based on all- versus-all BLASTp search with an E value of 1×10^{-3} , and clusters were defined with the Markov clustering algorithm and processed using vConTACT2 (Bin Jang et al., 2019). The stringency of the similarity score was evaluated through 1,000 randomizations by permuting protein clusters or singletons (proteins without significant shared similarity to other protein sequences) within pairs of sequences having a significance score ≤ 1 (negative control). Subsequently, pairs of

sequences with a similarity score > 1 were clustered into viral clusters with the Markov clustering algorithm using an inflation value of 2. The resulting gene-sharing network from vConTACT2 classification was visualized with Cytoscape software v. 3.7.0 (Smoot et al., 2011). Reference sequences from RefSeq database that co-clustered with the putative viral sequences were used to predict viral taxonomy.

Results

Site characteristics and taxonomic analysis

The sample site (Figure 1A and 1B) was a characteristic Namib Desert calcrete gravel plain (Gombeer et al., 2015) with high sand composition (92 ± 1.6 %) and very low organic carbon content (0.04%) (Supplementary Table S1). Soil chemical composition was relatively homogeneous in all sampled sectors and between the sample and control sites (Supplementary Table S1). Gravimetric water content measurements showed that more than half of the water content in the surface soil (0-5 cm) was lost 24 hours after the simulated 30 mm rainfall. After 3 days, the water content was similar to that of the dry control (Figure 1C).

Transcript read assembly yielded a consensus metatranscriptome of 208.95 Mb in which 378 802 coding regions were annotated, including 372 044 predicted protein-coding genes. On average, for the 24 sequenced libraries, 61.7% of reads could be aligned back to contigs. Functions were predicted for 29.9% of the protein-coding genes using the KEGG database (Kanehisa et al., 2016), and 51.6% using the CDD. 56.8% of contigs were taxonomically classified at phylum level, and 56.3% down to family level.

A functional and taxonomic analysis of the transcriptional profiles revealed large differences in gene expression levels between treatment and control soil transcriptomes within 10 minutes after watering (Figure 2, Supplementary Figure S2). Communities from dry soils were characterized by stable (i.e., largely unchanged) transcription over the 7-day experimental period. In contrast, microbial communities in the watered soil plot underwent an immediate and large change in gene expression (Figure 2A) that progressively returned to basal expression levels within 7 days from the wetting event. Taxonomy-only transcriptional profiles followed this cyclical pattern (Figure 2B) but were less robust than KO gene categories in representing the changes in the soil microbiome induced by soil wetting.

In both watered and dry soils, Actinobacteria and Proteobacteria were the most abundant phyla (average 40% and 16% in all samples, respectively, see Supplementary Table S2). Strikingly, transcripts from Nitrososphaeria, a class of ammonia oxidizing Thaumarchaeota, were the third most abundant (average 14% of classified TPM in all samples), suggesting that these taxa play an important role in the microbial community and in local nitrogen cycling. Protist transcripts (Oligohymenophorea class and Dictyostellales), which were rare in dry soil samples (<0.5%), increased to >4% within 7 hours of soil watering. Conversely, transcripts of fungal origin were more common in dry soils (1% of classified TPM) than in watered ones.

The production of ribosomes in each taxonomic group was analysed, as an indicator of their cellular metabolic activity, by quantifying ribosomal protein transcripts as a fraction of the total (Rp:T) (Bosdriesz et al., 2015; Bremer and Dennis, 1996). Ribosomal protein transcripts constituted a stable proportion for each taxon in

dry control samples, typically <4% TPM (Figure 3). After water addition, however, Rp:T increased for all major taxa, peaking at characteristic times over the course of the experiment and returning to basal values by the end of the 7 day period. Actinobacteria, Alpha-proteobacteria and Chloroflexi were examples of ‘early-activation’ groups, with Rp:T values peaking within the first hour after water addition. Gemmatimonadetes experienced the largest relative increase in Rp:T, despite being a minor component of the soil microbiome (<1% TPM). A delayed rise in activity, peaking at 7 hours after the simulated rainfall, was evident for Delta-proteobacteria, protists and Bacteroidetes, especially those belonging to the Cytophagia class, which constituted the principal ‘late-activation’ group. The Pezizomycetes, the most active fungal class, also showed a slower response to water addition. Intermediate response patterns, with maximal Rp:T values at 3 hours after water addition, were observed for taxa such as Planctomycetes. Thaumarchaeal Rp:T ratios showed only moderate changes after watering and a relatively even distribution throughout the experiment.

Temporal changes in core cellular functions

Watering induced a general reduction of stress resistance gene transcripts in the microbial community immediately after watering (e.g., *groEL*, *dnaK*, *clp*, *tre*). Trehalose synthesis genes (*tre*), which drive solute accumulation under conditions of water stress (Lebre et al., 2017), declined in relative transcription in watered soils. Transcripts for the major chaperones *groEL* and *dnaK*, and the *clp* protease involved in protein misfolding control were also reduced. The most conspicuous change in core stress resistance systems was, however, that of the heat-shock protein HSP20 genes (KO13993, CD223149 or CD278440). Genes for this ATP-independent chaperone

experienced the largest and most consistent transcript reduction across all significant (average ≥ 1 % of transcripts) bacterial taxa after watering (Figure 4), suggesting that HSP20 represents an archetypal "desiccation adaptation" gene.

Motility related transcripts were rapidly affected by soil wetting (from 10 minutes after water addition). Flagellar genes were up-regulated within the first hour in the dominant Actinobacteria and Alpha-proteobacteria (Figure 4) and in less dominant taxa such as Planctomycetes and Firmicutes. Type IV pilus genes, involved in gliding motility, were also rapidly upregulated after water addition, most notably in Actinobacteria. These responses suggest that rapid dispersal is a key behavioural response to soil wetting, particularly in Actinobacteria.

A significant up-regulation of Eukaryotic adhesion and cytoskeletal components, myosin, actin and tubulin, was evident 3 to 7 hours after soil wetting (Figure 4), but was limited to non-fungal taxa, particularly in Oligohymenophorea (ciliate) and Dictyosteliales (slime mould).

Another highly significant increase in transcriptional activity observed several hours after water addition involved interbacterial competition and predation genes. These included type VI secretion systems (T6SS) and vibriolysin encoding genes from the order Myxococcales (Delta-proteobacteria), and T6SS and serralyisin encoding genes from Alpha-proteobacteria. Heightened T6SS transcription was also observed in several other bacterial taxa, such as Planctomycetes, Gemmatimonadetes and Gamma-proteobacteria, suggesting that antagonistic interactions are of significant ecological importance in newly wetted soils.

Biogeochemical cycles

Carbon utilization: Despite the observation that microbial photosynthetic processes are water-limited in hyperarid soils (Tracy et al., 2010; Warren-Rhodes et al., 2006), transcript data did not show a significant increase in cyanobacterial gene expression after water addition, and both overall cyanobacterial transcript abundance and Rp:T ratios remained low (Figure 3, Supplementary Table S2). Furthermore, core carbon fixation genes, including RuBisCO and carboxylases genes from chemoautotrophic pathways, consistently showed reduced relative transcription in wetted soils. Thaumarchaeal carbon fixation was seemingly also affected by watering, reflected in the transient inhibition of 4-hydroxybutyryl-CoA dehydratase from the 3-hydroxypropionate/4-hydroxybutyrate cycle following inundation. These data are indicative of an inhibition of both photosynthetic and non-photosynthetic carbon fixation mechanisms immediately after soil saturation (Fig S3A). However, expression of carbon fixation genes increased by the end of the 7-day experimental period, when soil water content had returned to basal levels (Figure 4, Supplementary Figure S3A).

Dramatic increases in CO₂ emissions from newly wetted desert soils, attributed to degradation of dissolved organic matter, have been widely reported (Austin et al., 2004). Soil chemistry, however, showed no significant reduction in soil Total Organic Carbon (Supplementary Table S1), possibly due to limited sensitivity of the employed method at the very low values present in these soils. In our transcript data, indicators of biomass degradation, including carbohydrate and peptide transport systems (e.g., *rbsB*, *xylF*, and *livK*, *dppA*) significantly increased for most taxa immediately after water addition. The Bacteroidetes phylum, dominated by the Cytophagales, showed dramatic

up-regulation of subtilisin protease transcripts and components of the protein and carbohydrate import machinery (*ragA/susC* CD274948; *susD* CD185760), suggesting an important role of this group in the turnover of organic matter in the ecosystem.

Nitrogen and phosphate utilization: Transcript data suggested that Thaumarchaea were the main drivers of nitrogen cycling in dry soils. Ammonia monooxygenase and NO-forming nitrite reductase (*amo* and *nirK*) transcripts, originating largely from Thaumarchaea, were among the most abundant overall. Transcripts associated with both processes declined after soil watering (Figure 5A, Supplementary Figure S3B). All other detected transcripts implicated in ammonification, such as nitrate and nitrite reductases *narGH* and *nirAD* (mainly transcribed by Nitrospirae and Actinobacteria, respectively), and nitric oxide reductase (*norC*), were also reduced after watering. Conversely, peptide transporter transcripts (*livKH*, *dppA*) significantly increased in response to water addition for several taxa (Actinobacteria, Alpha-, Beta- and Delta-proteobacteria), suggesting that organic nitrogen dominates N acquisition processes in newly wetted soils.

Changes in phosphorus acquisition pathways were also identified. Transcript data suggested that phosphate acquisition after water addition was dominated by the proteobacterial *ugpB* sn-glycerol 3-phosphate (G3P) transport system subunit, particularly in Alpha-proteobacteria, followed by the *pst* inorganic phosphate transporter (Figure 5B). By contrast, phosphonate transport (*phn*) gene transcription was reduced after soil wetting.

Analysis of viral transcripts

A total of 68 contigs were characterized as being of viral origin using VirSorter (Supplementary Figure S4). In both dry and watered soil samples, viral contigs were significantly lower than bacterial contigs (ANOVA, $p < 0.01$), representing an average of $0.12 \pm 0.07\%$ TPM of viral protein transcripts. Over the period of the experiment, viral reads exhibited a significant temporal pattern, where read numbers remained low in all dry soil samples but exhibited a bimodal response in wetted soils (Figure 6). An initial very rapid increase in viral RNAs (approx. 2.2-fold at the first post-watering sample point: 10 min) was followed by a 12-fold decline over 7 hours. A second increase of viral read numbers (approx. 6-fold) occurred between 7 hours and 7 days. To investigate the diversity of the ‘active’ viral population, we used a genome-based network analysis of the shared protein content with the prokaryotic viral genomes (RefSeq v85). This analysis grouped 35 viral contigs into viral clusters (VCs) while 33 viral contigs remained non-clustered and were considered as singletons (Figure 7). In the network, 10 VCs containing viral contigs from our study were predicted, 7 of which did not belong to VCs with RefSeq virus genomes but instead clustered together into novel VCs, and 3 of which could be assigned taxonomy at the family level (Figure 7) as members of the *Caudovirales* (*Siphoviridae* and *Leviviridae*).

Discussion

Studies of the microbial ecology of desert edaphic niches have tended to focus on biological ‘hotspots’: hypoliths, soil crusts, and soils in the immediate vicinity of plants (e.g., Pointing and Belnap, 2012; Ramond et al., 2008; Marasco et al., 2018). While the use of high-throughput sequencing techniques (Crits-Christoph et al., 2013; Fierer et al., 2012, 2007; León-Sobrino et al., 2019; Jordaan et al., 2020; Vikram et al., 2016) has

greatly expanded of knowledge of the microbial ecology of these niches, most studies have centred on microbial diversity rather than functionality. Total RNA sequencing is therefore a valuable tool to monitor microbial functionality at a high temporal resolution, particularly since mRNA is only generated by active organisms and is ephemeral, leaving little to no legacy signal (León-Sobrinho et al., 2019; Rajeev et al., 2013; Steven et al., 2018).

Previous studies have clearly demonstrated a long-term compositional and functional adaptation of Namib Desert edaphic and hypolithic microbial communities to abiotic factors, particularly water input regimes histories (e.g., fog vs rain inputs; Cowan et al, 2020; Frossard et al., 2015; Ramond et al., 2018; Scola et al., 2018; Stomeo et al., 2013;). The location for this study site was situated in the central hyper-arid zone of the Namib Desert, which receives little input water from either rain or fog (Eckardt et al., 2013). The location should therefore optimise the responsiveness of the soil microbiome to water input (Seely et al., 2008).

The HSP20 chaperone is important for microbial life in desiccated soils

One of the projected effects of water addition was an apparent reduction in cellular stress, suggested by the immediate down-regulation in transcription of stress-resistance genes. The most conspicuous of these changes was the abrupt reduction in transcription - across all major bacterial taxa - of the small ATP-independent heat-shock protein HSP20. This chaperone has been characterized as a broad-spectrum bacterial stress resistance mechanism (Bepperling et al., 2012; Haslbeck and Vierling, 2015).

Desert soil microbial community members are sequentially activated after a water event

Data from the control (unwatered) site confirmed the presence of a diverse and functionally active microbial community in desiccated desert soils (see also, Gunnigle et al., 2017; Jordaan et al., 2020; León-Sobrino et al., 2019; Schulze-Makuch et al., 2018). However, our data suggest a level of remarkable "metabolic readiness", with a dramatic increase in transcription associated with previously inactive taxa occurred within 10 minutes after water addition. We note that transcriptional response rates may be considerably faster, given that 10 minutes was the first sampling time-point. In polyextreme hyperarid desert soils, where most (micro)organisms remain in a state of metabolic dormancy, such a rapid and opportunistic response to the sudden availability of water is clearly an adaptative advantage to access and utilize more favourable, and newly available, ecological substrates and niches.

Water addition led to a general increase in the relative abundance of ribosomal protein transcripts (Rp:T), interpreted as an increase in cellular activity (Bosdriesz et al., 2015; Bremer and Dennis, 1996). Cellular activity levels returned to basal (control) levels within 7 days, in parallel with the desiccation of the soil samples. These data are consistent with the paradigm that desert ecosystems and their indigenous microbiota are both resilient and water-pulse responsive (e.g., Belnap et al., 2005; Noy-Meir, 1973; Armstrong et al., 2016).

Interestingly, various groups of taxa reached maximum Rp:T values at different times, suggesting a controlled pattern of functionality reminiscent of a stepwise model where ecosystem functions gradually evolve as a function of the duration and intensity

of the water pulses (Schwinning and Sala, 2004). The most immediate microbial response was characterized by transcription of genes implicated in the motility apparatus (type IV pili in Alpha-proteobacteria and flagella in Actinobacteria). It has been previously noted that one of the main impacts of water inundation is increased soil particle connectivity, providing access to new niches and solubilized nutrients (Schimel, 2018). Actinobacteria and Alpha-proteobacteria have been reported as the dominant active taxa in desiccated soils (León-Sobrinho et al., 2019), possibly uniquely positioned to access new and more favourable niches during periods of inter-connection associated with the water-saturated state.

A significant, but delayed, transcriptional activation was observed in the non-fungal microbial eukaryotes (e.g., protists) and Deltaproteobacteria; i.e., 3 to 7 hours after water addition. The former were mostly characterized by structural gene transcripts from the cytoskeleton, a generic indication of overall cellular activity (cell motility and/or cell division). Up-regulated Deltaproteobacterial transcripts were predominantly derived from the Myxococcales, an order of well-known predatory bacteria (Jurkevitch and Davidov, 2007; Shimkets et al., 2006). The dramatic increase in protist and myxobacterial activity is strongly suggestive of predatory behaviour (Thiery and Kaimer, 2020), possibly triggered by increases in prey abundance (i.e., Actinobacteria and Alphaproteobacteria populations) rather than just by soil rehydration.

Namib Desert fungal and cyanobacterial soil populations are not activated by water

Among the edaphic taxa that were essentially non-responsive to water addition, we particularly identified the Cyanobacteria and most Fungi, with almost none of their genes being differentially up-regulated at a statistically significant level.

We anticipated that water addition would trigger a significant and rapid increase in primary production markers linked to cyanobacterial and photosynthetic activity, as previously observed in biological soil crusts and hypolithic communities (Angel and Conrad, 2013; Pringault and Garcia-Pichel, 2004; Rajeev et al., 2013; Steven et al., 2018; Warren-Rhodes et al., 2006). The almost complete absence of water input-related activation of cyanobacterial functionality suggests that primary productivity in hyperarid soils may not be driven by cyanobacteria and is consistent with previous observations showing that hypolithons (and maybe other cryptic communities) are the foundation of productivity after rain events in the Namib Desert (Ramond et al., 2018).

Desert edaphic viral communities change over time after a wetting event

The extent to which microbial communities in desert soils are influenced by phage remains largely unexplored (Fancello et al., 2013; Zablocki et al., 2016; 2017). This study provides one of the first temporal assessments of active phage in a desert edaphic environment (Zablocki et al., 2016). In parallel with the rapid changes in bacterial metatranscriptomic patterns, the phage population responded within 10 min after water addition, followed by a sharp decrease and a secondary increase broadly correlating with the 'late response' fraction of the soil bacterial community.

We propose two possible mechanisms which could lead to the bimodal response of viral communities in wetted soils: a direct response to increased predation and/or a

kill-the-winner model as observed in aquatic ecosystems (e.g., Shapiro et al., 2010). Microbial taxa in a 'biologically hostile' environment may be forced to allocate resources to energetically expensive defence mechanisms, potentially resulting in lowered defence against phage and elevated susceptibility to infection (Chen and Williams, 2012; Friman and Buckling, 2013; Örmälä-Odegrip et al., 2015). The bacterial host's capacity to maintain the 'arms race' dynamic (i.e., co-escalation of host resistance and parasite infectivity) may also be degraded in the presence of predators (Friman and Buckling, 2013).

We note that most of the viral sequences identified in this study were unclassified suggesting, as previously observed (Scola et al., 2018), that hot desert soils may harbour a substantial and potentially novel phage genomic and taxonomic diversity.

Water pulses shift microbial C, N and P nutrient utilization patterns

It was anticipated that water addition would trigger a significant increase in primary productivity, since photosynthetic processes are highly sensitive to water activity (Brock, 1975; Steven et al., 2018; Warren-Rhodes et al., 2006). However, markers for photosynthetic and chemoautotrophic carbon fixation (the latter being active in desiccated periods (León-Sobrino et al., 2019; Sghaier et al., 2016)), were significantly suppressed. This suggests that open soil communities may be dependent on carbon input from alternative sources, such as sporadic vegetation growth and/or productive cryptic niches such as hypolithons and biological soil crusts (Armstrong et al., 2016; Ramond et al., 2018). Nitrogen cycling genes, particularly those involved in inorganic nitrogen acquisition (i.e., nitrate, via nitrate reductases), were also downregulated after water addition. This was also surprising as active N mineralization

and N loss processes are often increased in arid soils in response to rainfall (Austin et al., 2004), and desert soils are particularly rich in nitrate (Graham et al., 2008; Walwoord et al., 2003). However, a metabolic switch to nitrogen acquisition from organic substrates was strongly suggested by the upregulation of peptide transporter genes, mirroring the situation observed for carbon acquisition. The transient reduction in autotrophic C and N fixation after watering may be explained in terms of energy efficiency, where the sudden availability of 'energy-rich' substrates provides a favoured heterotrophic resource over energetically expensive autotrophic processes (Fuchs, 2011).

The addition of water triggered an upregulation of genes involved in inorganic phosphate transport and a downregulation of those implicated in organic phosphonate acquisition. We speculate that the solubilization of inorganic phosphate from soil particles displaces phosphonates as the preferred P source (Schowanek and Verstraete, 1990). Noticeable exceptions were the Alpha-proteobacterial taxa, which apparently favour organic G3P as a preferred P source, both in desiccated soils and after wetting (León-Sobrino et al., 2019).

A conceptual response model of desert soil edaphic microbial communities to water

From a composite analysis of our metatranscriptomic data, we propose a rainfall response model for desert soil microbiomes (Figure 8). Immediately after soil wetting (≤ 10 min), some bacterial taxa (particularly Actinobacteria and Alpha-proteobacteria, that show significant chemoautotrophic capacity in dry soils (León-Sobrino et al., 2019)) reduce autotrophic carbon fixation activities, activate cellular uptake mechanisms and engage in dispersal using both natatory (flagella) and gliding (type IV

pili) mechanisms, presumably in order to colonize new niches and access new substrate resource pools. There is a concomitant increase in active phage particles.

Following this rapid dispersal burst, approximately 3 to 7 hours after water addition predatory and saprophytic microbial taxa are activated. These predators include eukaryotes, especially ciliates (Oligohymenophorea class), Dictyostellid amoebae, Delta-proteobacteria (myxobacteria) and Bacteroidetes (Cytophagia class). Simultaneously, and presumably in response to the activation of predators, several bacterial groups upregulate the transcription of defensive systems, most notably T6SS. A second wave of viral infections appears to follow the emergence of eukaryotic predators.

In the final stage of the wetting-drying cycle (approx. 7 days after water addition), when soils are effectively dehydrated to pre-watering levels, autotrophic carbon fixation processes and nitrogen cycling are reactivated in the bacterial community, along with certain desiccation stress resistance mechanisms, most especially HSP20.

Conclusion

In this 7 day *in situ* metatranscriptomics study, we have observed the dominant microbial processes induced by ‘rainfall’ on hyperarid soils and successfully followed a complete cycle of microbial activation and subsequent inactivation as soils and their microbial communities returned to their basal desiccated state and transcriptional status. We show strong evidence of short-term temporal succession and, by implication, tightly regulated processes in desert edaphic communities after water addition, which favour heterotrophy over autotrophy. Furthermore, we suggest that

dispersal-predation cycles, in which both phage and predators play active roles, are important driving forces in shaping community composition within a wetting-drying regime in hyper-arid desert soils.

Declarations

Acknowledgements

The authors wish to thank the National Research Foundation of South Africa and the University of Pretoria for financial support, and the staff and students of the Gobabeb-Namib Research Institute for field support.

Funding

The authors acknowledge funding support from the University of Pretoria and the South African National Research Foundation (grant number 113308).

Data availability

The dataset supporting the conclusions of this article is available in the ArrayExpress repository, <https://www.ebi.ac.uk/arrayexpress/> (accession no. E-MTAB-9439). Reference metatranscriptome assembly and annotations can be accessed at the IMG/M repository, <https://img.jgi.doe.gov/> (GOLD Analysis Project Id Ga0326365)

Competing interests

The authors declare no competing financial interests and no conflict of interest.

Author contributions

C. L.-S., J.-B. R. and D. A. C. conceived the experiment. C. L.-S., J.-B. R. and R. K. performed the sample collection. C. L.-S. performed all experimental work. C. L.-S. and C. C. performed the microbial and viral computational data analysis, respectively. C. L.-

S., C. C., J.-B. R. and D. A. C. participated in the interpretation of results and writing of the manuscript. G. M.-K. provided logistical support and field advice in the Namib Desert.

References

- Adriaenssens EM, Kramer R, Van Goethem MW, Makhalanyane TP, Hogg I, Cowan DA. 2017. Environmental drivers of viral community composition in Antarctic soils identified by viromics. *Microbiome* 5:83. doi:10.1186/s40168-017-0301-7
- Angel R, Conrad R. 2013. Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. *Environ Microbiol* 15:n/a-n/a. doi:10.1111/1462-2920.12140
- Armstrong A, Valverde A, Ramond JB, Makhalanyane TP, Jansson JK, Hopkins DW, Aspray TJ, Seely M, Trindade MI, Cowan DA. 2016. Temporal dynamics of hot desert microbial communities reveal structural and functional responses to water input. *Sci Rep* 6:34434. doi:10.1038/srep34434
- Austin AT, Yahdjian L, Stark JM, Belnap J, Porporato A, Norton U, Ravetta DA, Schaeffer SM. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia* 141:221–235. doi:10.1007/s00442-004-1519-1
- Bär M, Hardenberg J, Meron E, Provenzale A. 2002. Modelling the survival of bacteria in drylands: the advantage of being dormant. *Proc R Soc London Ser B Biol Sci* 269:937–942. doi:10.1098/rspb.2002.1958
- bbmap. 2018. Masked version of hg19 by Brian Bushnell.

516 doi:10.5281/ZENODO.1208052

517 Belnap J, Welter JR, Grimm NB, Barger N, Ludwig JA. 2005. Linkages between microbial
518 and hydrologic processes in arid and semiarid watersheds. *Ecology* **86**:298–307.
519 doi:10.1890/03-0567

520 Bepperling A, Alte F, Kriehuber T, Braun N, Weinkauff S, Groll M, Haslbeck M, Buchner J.
521 2012. Alternative bacterial two-component small heat shock protein systems.
522 *Proc Natl Acad Sci* 109:20407–20412. doi:10.1073/pnas.1209565109

523 Bezuidt OKI, Lebre PH, Pierneef R, León-Sobrino C, Adriaenssens EM, Cowan DA, Van
524 de Peer Y, Makhalanyane TP. 2020. Phages Actively Challenge Niche Communities
525 in Antarctic Soils. *mSystems* 5:e00234-20. doi:10.1128/mSystems.00234-20

526 Bin Jang H, Bolduc B, Zablocki O, Kuhn JH, Roux S, Adriaenssens EM, Brister JR,
527 Kropinski AM, Krupovic M, Lavigne R, Turner D, Sullivan MB. 2019. Taxonomic
528 assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing
529 networks. *Nat Biotechnol* 37:632–639. doi:10.1038/s41587-019-0100-8

530 Bolduc B, Youens-Clark K, Roux S, Hurwitz BL, Sullivan MB. 2017. IVirus: Facilitating
531 new insights in viral ecology with software and community data sets imbedded in
532 a cyberinfrastructure. *ISME J* 11:7–14. doi:10.1038/ismej.2016.89

533 Boratyn GM, Schäffer AA, Agarwala R, Altschul SF, Lipman DJ, Madden TL. 2012.
534 Domain enhanced lookup time accelerated BLAST. *Biol Direct* 7:12.
535 doi:10.1186/1745-6150-7-12

536 Bosdriesz E, Molenaar D, Teusink B, Bruggeman FJ. 2015. How fast-growing bacteria

537 robustly tune their ribosome concentration to approximate growth-rate
538 maximization. FEBS J 282:2029–44. doi:10.1111/febs.13258

539 Bremer H, Dennis P. 1996. Modulation of chemical composition and other parameters
540 of the cell by growth rate. *Escherichia coli Salmonella* Cell Mol Biol 2.

541 Brock TDT. 1975. Effect of water potential on a *Microcoleus* (Cyanophyceae) from a
542 desert crust. J Phycol 11:316–320. doi:10.1111/j.1529-8817.1975.tb02786.x

543 Bushnell B, Rood J, Singer E. 2017. BBMerge – Accurate paired shotgun read merging
544 via overlap. PLoS One 12:e0185056. doi:10.1371/journal.pone.0185056

545 Chen H, Williams HN. 2012. Sharing of prey: coinfection of a bacterium by a virus and a
546 prokaryotic predator. MBio 3:4–6. doi:10.1128/mBio.00051-12

547 Collins SL, Belnap J, Grimm NB, Rudgers JA, Dahm CN, D’Odorico P, Litvak M, Natvig
548 DO, Peters DC, Pockman WT, Sinsabaugh RL, Wolf BO. 2014. A multiscale,
549 hierarchical model of pulse dynamics in arid-land ecosystems. Annu Rev Ecol Evol
550 Syst 45:397–419. doi:10.1146/annurev-ecolsys-120213-091650

551 Cowan DA, Hopkins DW, Jones BE, Maggs-Kölling G, Majewska R, Ramond J-B. 2020.
552 Microbiomics of namib desert habitats. *Extremophiles* 24:17–29.
553 doi:10.1007/s00792-019-01122-7

554 Crits-Christoph A, Robinson CK, Barnum T, Fricke WF, Davila AF, Jedynak B, McKay CP,
555 Diruggiero J. 2013. Colonization patterns of soil microbial communities in the
556 Atacama Desert. Microbiome 1:28. doi:10.1186/2049-2618-1-28

557 Delgado-Baquerizo M, Maestre FT, Gallardo A, Bowker MA, Wallenstein MD, Quero JL,

558 Ochoa V, Gozalo B, García-Gómez M, Soliveres S, García-Palacios P, Berdugo M,
559 Valencia E, Escolar C, Arredondo T, Barraza-Zepeda C, Bran D, Carreira JA, Chaieb
560 M, Conceicao AA, Derak M, Eldridge DJ, Escudero A, Espinosa CI, Gaitán J, Gatica
561 MG, Gómez-González S, Guzman E, Gutiérrez JR, Florentino A, Hepper E,
562 Hernández RM, Huber-Sannwald E, Jankju M, Liu J, Mau RL, Miriti M, Monerris J,
563 Naseri K, Noumi Z, Polo V, Prina A, Pucheta E, Ramírez E, Ramírez-Collantes DA,
564 Romao R, Tighe M, Torres D, Torres-Díaz C, D. Ungar E, Val J, Wamiti W, Wang D,
565 Zaady E. 2013. Decoupling of soil nutrient cycles as a function of aridity in global
566 drylands. *Nature* 502:672–676. doi:10.1038/nature12670

567 Delgado-Baquerizo M, Maestre FT, Reich PB, Jeffries TC, Gaitan JJ, Encinar D, Berdugo
568 M, Campbell CD, Singh BK. 2016. Microbial diversity drives multifunctionality in
569 terrestrial ecosystems. *Nat Commun* 7:10541. doi:10.1038/ncomms10541

570 Eckardt FD, Soderberg K, Coop LJ, Muller AA, Vickery KJ, Grandin RD, Jack C, Kapalanga
571 TS, Henschel J. 2013. The nature of moisture at Gobabeb, in the central Namib
572 Desert. *J Arid Environ* 93:7–19. doi:10.1016/j.jaridenv.2012.01.011

573 Fancello L, Trape S, Robert C, Boyer M, Popgeorgiev N, Raoult D, Desnues C. 2013.
574 Viruses in the desert: a metagenomic survey of viral communities in four
575 perennial ponds of the Mauritanian Sahara. *ISME J* 7:359–69.
576 doi:10.1038/ismej.2012.101

577 Fierer N, Breitbart M, Nulton J, Salamon P, Lozupone C, Jones R, Robeson M, Edwards
578 RA, Felts B, Rayhawk S, Knight R, Rohwer F, Jackson RB. 2007. Metagenomic and
579 small-subunit rRNA analyses reveal the genetic diversity of bacteria, archaea,

580 fungi, and viruses in soil. Appl Environ Microbiol 73:7059–66.
581 doi:10.1128/AEM.00358-07

582 Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall
583 DH, Caporaso JG. 2012. Cross-biome metagenomic analyses of soil microbial
584 communities and their functional attributes. Proc Natl Acad Sci U S A 109:21390–
585 5. doi:10.1073/pnas.1215210110

586 Friman VP, Buckling A. 2013. Effects of predation on real-time host-parasite
587 coevolutionary dynamics. Ecol Lett 16:39–46. doi:10.1111/ele.12010

588 Frossard A, Ramond J-B, Seely M, Cowan DA. 2015. Water regime history drives
589 responses of soil Namib Desert microbial communities to wetting events. Sci Rep
590 5:12263. doi:10.1038/srep12263

591 Fuchs G. 2011. Alternative pathways of carbon dioxide fixation: Insights into the early
592 evolution of life? Annu Rev Microbiol 65:631–658. doi:10.1146/annurev-micro-
593 090110-102801

594 Garcia-Pichel F, Pringault O. 2001. Cyanobacteria track water in desert soils. Nature
595 413:380–381. doi:10.1038/35096640

596 Gee GW, Bauder JW. 1986. Particle-size analysis In: Klute A, editor. Methods of Soil
597 Analysis: Part 1—Physical and Mineralogical Methods, SSSA Book Series SV - 5.1.
598 Madison, WI: Soil Science Society of America, American Society of Agronomy. pp.
599 383–411. doi:10.2136/sssabookser5.1.2ed.c15

600 Gombeer S, Ramond J-B, Eckardt FD, Seely M, Cowan DA. 2015. The influence of

601 surface soil physicochemistry on the edaphic bacterial communities in contrasting
602 terrain types of the Central Namib Desert. *Geobiology* 13:494–505.
603 doi:10.1111/gbi.12144

604 Graham RC, Hirmas DR, Wood YA, Amrhein C. 2008. Large near-surface nitrate pools in
605 soils capped by desert pavement in the Mojave Desert, California. *Geology*
606 **36**:259–262. doi:10.1130/G24343A.1

607 Gunnigle E, Frossard A, Ramond J-B, Guerrero L, Seely M, Cowan DA. 2017. Diel-scale
608 temporal dynamics recorded for bacterial groups in Namib Desert soil. *Sci Rep*
609 **7**:40189. doi:10.1038/srep40189

610 Haslbeck M, Vierling E. 2015. A first line of stress defense: small heat shock proteins
611 and their function in protein homeostasis. *J Mol Biol* 427:1537–1548.
612 doi:10.1016/J.JMB.2015.02.002

613 Huang J, Yu H, Guan X, Wang G, Guo R. 2016. Accelerated dryland expansion under
614 climate change. *Nat Clim Chang* 6:166–171. doi:10.1038/nclimate2837

615 Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K,
616 Szeto E, Pillay M, Chen I-MA, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015.
617 The standard operating procedure of the DOE-JGI Microbial Genome Annotation
618 Pipeline (MGAP v.4). *Stand Genomic Sci* 10:86. doi:10.1186/s40793-015-0077-y

619 Hyatt D, Chen G-L, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal:
620 prokaryotic gene recognition and translation initiation site identification. *BMC*
621 *Bioinformatics* 11:119. doi:10.1186/1471-2105-11-119

622 Jacobson K, Van Diepeningen A, Evans S, Fritts R, Gemmel P, Marsho C, Seely M,
623 Wenndt A, Yang X, Jacobson P. 2015. Non-rainfall moisture activates fungal
624 decomposition of surface litter in the Namib Sand Sea. PLoS One 10:e0126977.
625 doi:10.1371/journal.pone.0126977

626 Johnson RM, Ramond J-B, Gunnigle E, Seely M, Cowan DA. 2017. Namib Desert
627 edaphic bacterial, fungal and archaeal communities assemble through
628 deterministic processes but are influenced by different abiotic parameters.
629 Extremophiles 21:381–392. doi:10.1007/s00792-016-0911-1

630 Jordaan K, Lappan R, Dong X, Aitkenhead IJ, Bay SK, Chiri E, Wieler N, Meredith LK,
631 Cowan DA, Chown SL, Greening C. 2020. Hydrogen-oxidizing bacteria are
632 abundant in desert soils and strongly stimulated by hydration. *mSystems*
633 5:e01131-20. doi:10.1128/mSystems.01131-20

634 Jurkevitch E, Davidov Y. 2007. Phylogenetic diversity and evolution of predatory
635 prokaryotes In: Heidelberg, editor. *Predatory Prokaryotes, Microbiology*
636 *Monographs*. Berlin: Springer Berlin Heidelberg. pp. 11–56.
637 doi:10.1007/7171_052

638 Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. 2016. KEGG as a reference
639 resource for gene and protein annotation. *Nucleic Acids Res* 44:D457-62.
640 doi:10.1093/nar/gkv1070

641 Laity JJ. 2009. *Deserts and desert environments*, Vol. 3. ed. Chichester, UK: Wiley-
642 Blackwell.

643 Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat
644 Methods 9:357–359. doi:10.1038/nmeth.1923

645 Lebre PH, De Maayer P, Cowan DA. 2017. Xerotolerant bacteria: surviving through a
646 dry spell. Nat Rev Microbiol 15:285–296. doi:10.1038/nrmicro.2017.16

647 León-Sobrino C, Ramond J-BBJ-B, Maggs-Kölling G, Cowan DADADA. 2019. Nutrient
648 acquisition, rather than stress response over diel cycles, drives microbial
649 transcription in a hyper-arid namib desert soil. Front Microbiol 10:1054.
650 doi:10.3389/fmicb.2019.01054

651 Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin
652 R. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics
653 25:2078–2079. doi:10.1093/bioinformatics/btp352

654 Liao Y, Smyth GK, Shi W. 2014. FeatureCounts: An efficient general purpose program
655 for assigning sequence reads to genomic features. Bioinformatics 30:923–930.
656 doi:10.1093/bioinformatics/btt656

657 Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and
658 dispersion for RNA-seq data with DESeq2. Genome Biol 15:550.
659 doi:10.1186/s13059-014-0550-8

660 Luo E, Eppley JM, Romano AE, Mende DR, DeLong EF. 2020. Double-stranded DNA
661 viroplankton dynamics and reproductive strategies in the oligotrophic open
662 ocean water column. ISME J 14:1304–1315. doi:10.1038/s41396-020-0604-8

663 Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B, Quero

664 JL, García-Gómez M, Gallardo A, Ulrich W, Bowker MA, Arredondo T, Barraza-
665 Zepeda C, Bran D, Florentino A, Gaitán J, Gutiérrez JR, Huber-Sannwald E, Jankju
666 M, Mau RL, Miriti M, Naseri K, Ospina A, Stavi I, Wang D, Woods NN, Yuan X,
667 Zaady E, Singh BK. 2015. Increasing aridity reduces soil microbial diversity and
668 abundance in global drylands. *Proc Natl Acad Sci U S A* 112:15684–9.
669 doi:10.1073/pnas.1516684112

670 Mandakovic D, Rojas C, Maldonado J, Latorre M, Trivisany D, Delage E, Bihouée A,
671 Jean G, Díaz FP, Fernández-Gómez B, Cabrera P, Gaete A, Latorre C, Gutiérrez RA,
672 Maass A, Cambiazo V, Navarrete SA, Eveillard D, González M. 2018. Structure and
673 co-occurrence patterns in microbial communities under acute environmental
674 stress reveal ecological factors fostering resilience. *Sci Rep* 8:0–12.
675 doi:10.1038/s41598-018-23931-0

676 Marasco R, Mosqueira MJ, Fusi M, Ramond J-B, Merlino G, Booth JM, Maggs-Köling G,
677 Cowan DA, Daffonchio D. 2018. Rhizosphere microbial community assembly of
678 sympatric desert speargrasses is independent of the plant host. *Microbiome*
679 6:215. doi:10.1186/s40168-018-0597-y

680 Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, Geer RC, He J,
681 Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z,
682 Yamashita RA, Zhang D, Zheng C, Bryant SH. 2015. CDD: NCBI's conserved domain
683 database. *Nucleic Acids Res* 43:D222–D226. doi:10.1093/nar/gku1221

684 McHugh TA, Compson Z, Gestel N van, Hayer M, Ballard L, Haverty M, Hines J, Irvine N,
685 Krassner D, Lyons T, Musta EJ, Schiff M, Zint P, Schwartz E. 2017. Climate controls

686 prokaryotic community composition in desert soils of the southwestern United
687 States. FEMS Microbiol Ecol 93:1–13. doi:10.1093/femsec/fix116

688 Oren N, Raanan H, Murik O, Keren N, Kaplan A. 2017. Dawn illumination prepares
689 desert cyanobacteria for dehydration. Curr Biol 27:R1056–R1057.
690 doi:10.1016/j.cub.2017.08.027

691 Örmälä-Odegrip AM, Ojala V, Hiltunen T, Zhang J, Bamford JK, Laakso J. 2015. Protist
692 predation can select for bacteria with lowered susceptibility to infection by lytic
693 phages Experimental evolution. BMC Evol Biol 15:1–7. doi:10.1186/s12862-015-
694 0341-1

695 Palmer FE, Emery DR, Stemmler J, Staley JT. 1987. Survival and growth of microcolonial
696 rock fungi as affected by temperature and humidity. New Phytol 107:155–162.
697 doi:10.1111/j.1469-8137.1987.tb04889.x

698 Pointing SBBB, Belnap J. 2012. Microbial colonization and controls in dryland systems.
699 Nat Rev Microbiol 10:551–562. doi:10.1038/nrmicro2831

700 Pringault O, Garcia-Pichel F. 2004. Hydrotaxis of Cyanobacteria in Desert Crusts.
701 Microb Ecol 47:366–73. doi:10.1007/s00248-002-0107-3

702 Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO.
703 2012. The SILVA ribosomal RNA gene database project: improved data processing
704 and web-based tools. Nucleic Acids Res 41:D590–D596. doi:10.1093/nar/gks1219

705 Rajeev L, Da Rocha UNN, Klitgord N, Luning EGG, Fortney J, Axen SDD, Shih PMM,
706 Bouskill NJJ, Bowen BPP, Kerfeld CAA, Garcia-Pichel F, Brodie ELL, Northen TRR,

707 Mukhopadhyay A. 2013. Dynamic cyanobacterial response to hydration and
708 dehydration in a desert biological soil crust. *ISME J* 7:2178–2191.
709 doi:10.1038/ismej.2013.83

710 Ramond J-B, Woodborne S, Hall G, Seely M, Cowan DA. 2018. Namib Desert primary
711 productivity is driven by cryptic microbial community N-fixation. *Sci Rep* 8:6921.
712 doi:10.1038/s41598-018-25078-4

713 Robertson G, Schein J, Chiu R, Corbett R, Field M, Jackman SDD, Mungall K, Lee S,
714 Okada HMM, Qian JQQ, Griffith M, Raymond A, Thiessen N, Cezard T, Butterfield
715 YSS, Newsome R, Chan SKK, She R, Varhol R, Kamoh B, Prabhu A-LAL, Tam A, Zhao
716 Y, Moore RAA, Hirst M, Marra MAA, Jones SJM, Hoodless PAA, Birol I. 2010. *De*
717 *novo* assembly and analysis of RNA-seq data. *Nat Methods* 7:909–912.
718 doi:10.1038/nmeth.1517

719 Rodriguez-Valera F, Martin-Cuadrado AB, Rodriguez-Brito B, Pašić L, Thingstad TF,
720 Rohwer F, Mira A. 2009. Explaining microbial population genomics through phage
721 predation. *Nat Rev Microbiol* 7:828–836. doi:10.1038/nrmicro2235

722 Ronca S, Ramond J-B, Jones BE, Seely M, Cowan DA. 2015. Namib Desert
723 dune/interdune transects exhibit habitat-specific edaphic bacterial communities.
724 *Front Microbiol* 6:845. doi:10.3389/fmicb.2015.00845

725 Roux S, Enault F, Hurwitz BL, Sullivan MB. 2015. VirSorter: Mining viral signal from
726 microbial genomic data. *PeerJ* 2015:1–20. doi:10.7717/peerj.985

727 Schimel JP. 2018. Life in Dry Soils: Effects of Drought on Soil Microbial Communities

728 and Processes. *Annu Rev Ecol Evol Syst* 49:409–432. doi:10.1146/annurev-ecolsys-
729 110617-062614

730 Schlesinger WH, Raikes JA, Hartley AE, Cross AF. 1995. On the spatial pattern of soil
731 nutrients in desert ecosystems. *Ecology* 77:364–374. doi:10.2307/2265615

732 Schowanek D, Verstraete W. 1990. Phosphonate utilization by bacteria in the presence
733 of alternative phosphorus sources. *Biodegradation* 1:43–53.
734 doi:10.1007/BF00117050

735 Schulze-Makuch D, Wagner D, Kounaves SP, Mangelsdorf K, Devine KG, de Vera J-P,
736 Schmitt-Kopplin P, Grossart H-P, Parro V, Kaupenjohann M, Galy A, Schneider B,
737 Airo A, Frösler J, Davila AF, Arens FL, Cáceres L, Cornejo FS, Carrizo D, Dartnell L,
738 DiRuggiero J, Flury M, Ganzert L, Gessner MO, Grathwohl P, Guan L, Heinz J, Hess
739 M, Keppler F, Maus D, McKay CP, Meckenstock RU, Montgomery W, Oberlin EA,
740 Probst AJ, Sáenz JS, Sattler T, Schirmack J, Sephton MA, Schlöter M, Uhl J,
741 Valenzuela B, Vestergaard G, Wörmer L, Zamorano P. 2018. Transitory microbial
742 habitat in the hyperarid Atacama Desert. *Proc Natl Acad Sci U S A* 115:2670–2675.
743 doi:10.1073/pnas.1714341115

744 Schwinning S, Sala OE. 2004. Hierarchy of responses to resource pulses in arid and
745 semi-arid ecosystems. *Oecologia* 141:211–220. doi:10.1007/s00442-004-1520-8

746 Scola V, Ramond J-B, Frossard A, Zablocki O, Adriaenssens EM, Johnson RM, Seely M,
747 Cowan DA. 2018. Namib Desert soil microbial community diversity, assembly, and
748 function along a natural xeric gradient. *Microb Ecol* 75:193–203.
749 doi:10.1007/s00248-017-1009-8

750 Seely M, Pallett J, Pallet J. 2008. Namib: Secrets of a desert uncovered, Venture. ed.
751 Windhoek, Namibia, Namibia: Venture Publications.

752 Sghaier H, Hezbri K, Ghodhbane-Gtari F, Pujic P, Sen A, Daffonchio D, Boudabous A,
753 Tisa LS, Klenk H-P, Armengaud J, Normand P, Gtari M. 2016. Stone-dwelling
754 actinobacteria *Blastococcus saxobsidens*, *Modestobacter marinus* and
755 *Geodermatophilus obscurus* proteogenomes. ISME J 10:21–29.
756 doi:10.1038/ismej.2015.108

757 Shapiro OH, Kushmaro A, Brenner A. 2010. Bacteriophage predation regulates
758 microbial abundance and diversity in a full-scale bioreactor treating industrial
759 wastewater. *ISME J* 4:327–336. doi:10.1038/ismej.2009.118

760 Shimkets L, Dworkin M, Reichenbach H. 2006. The Myxobacteria BT In: Dworkin M,
761 Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E, editors. The Prokaryotes:
762 Volume 7: Proteobacteria: Delta, Epsilon Subclass. New York, NY. pp. 31–115.

763 Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. 2011. Cytoscape 2.8: New features
764 for data integration and network visualization. *Bioinformatics* 27:431–432.
765 doi:10.1093/bioinformatics/btq675

766 Steven B, Belnap J, Kuske CR. 2018. Chronic physical disturbance substantially alters
767 the response of biological soil crusts to a wetting pulse, as characterized by
768 metatranscriptomic sequencing. *Front Microbiol* 9:2382.
769 doi:10.3389/fmicb.2018.02382

770 Stomeo F, Valverde A, Pointing SB, McKay CP, Warren-Rhodes KA, Tuffin MI, Seely M,

771 Cowan DA. 2013. Hypolithic and soil microbial community assembly along an
772 aridity gradient in the Namib Desert. *Extremophiles* 17:329–37.
773 doi:10.1007/s00792-013-0519-7

774 Štoviček A, Kim M, Or D, Gillor O. 2017. Microbial community response to hydration-
775 desiccation cycles in desert soil. *Sci Rep* 7:45735. doi:10.1038/srep45735

776 Szymanski M, Zielezinski A, Barciszewski J, Erdmann VA, Karlowski WM. 2016.
777 5SRNAdb: an information resource for 5S ribosomal RNAs. *Nucleic Acids Res*
778 44:D180–D183. doi:10.1093/nar/gkv1081

779 Thiery S, Kaimer C. 2020. The predation strategy of *Myxococcus xanthus*. *Front*
780 *Microbiol* 11:2. doi:10.3389/fmicb.2020.00002

781 Tracy CR, Streten-Joyce C, Dalton R, Nussear KE, Gibb KS, Christian KA. 2010.
782 Microclimate and limits to photosynthesis in a diverse community of hypolithic
783 cyanobacteria in northern Australia. *Environ Microbiol* 12:592–607.
784 doi:10.1111/j.1462-2920.2009.02098.x

785 Trubl G, Hyman P, Roux S. 2020. Coming-of-age characterization of soil viruses: a user's
786 guide to virus isolation, detection within metagenomes, and viromics. *Soil systems*
787 4:1–34.

788 Trubl G, Roux S, Solonenko N, Li Y-F, Bolduc B, Rodríguez-Ramos J, Eloë-Fadrosh EA,
789 Rich VI, Sullivan MB. 2019. Towards optimized viral metagenomes for double-
790 stranded and single-stranded DNA viruses from challenging soils. *PeerJ* 7:e7265.
791 doi:10.7717/peerj.7265

792 van der Walt AJJ, Johnson RMM, Cowan DAA, Seely M, Ramond J-BJB. 2016. Unique
793 microbial phylotypes in namib desert dune and gravel plain fairy circle soils. *Appl*
794 *Environ Microbiol* 82:4592–601. doi:10.1128/AEM.00844-16

795 Vikram S, Guerrero LD, Makhalanyane TP, Le PT, Seely M, Cowan DA. 2016.
796 Metagenomic analysis provides insights into functional capacity in a hyperarid
797 desert soil niche community. *Environ Microbiol* 18:1875–1888. doi:10.1111/1462-
798 2920.13088

799 Walkley A. 1935. An examination of methods for determining organic carbon and
800 nitrogen in soils. *J Agric Sci* 25:598–609. doi:10.1017/S0021859600019687

801 Walvoord MA. 2003. A reservoir of nitrate beneath desert soils. *Science* (80-)
802 **302**:1021–1024. doi:10.1126/science.1086435

803 Warren-Rhodes KA, Rhodes KL, Pointing SB, Ewing SA, Lacap DC, Gómez-Silva B,
804 Amundson R, Friedmann EI, McKay CP. 2006. Hypolithic cyanobacteria, dry limit
805 of photosynthesis, and microbial ecology in the hyperarid Atacama Desert.
806 *Microb Ecol* 52:389–398. doi:10.1007/s00248-006-9055-7

807 Williamson KE, Fuhrmann JJ, Wommack KE, Radosevich M. 2017. Viruses in soil
808 ecosystems: an unknown quantity within an unexplored territory. *Annu Rev Virol*
809 **4**:201–219. doi:10.1146/annurev-virology-101416-041639

810 Zablocki O, Adriaenssens EM, Cowan D. 2016. Diversity and ecology of viruses in
811 hyperarid desert soils. *Appl Environ Microbiol* **82**:770–777.
812 doi:10.1128/AEM.02651-15

813 Zablocki O, Adriaenssens EM, Frossard A, Seely M, Ramond JB, Cowan D. 2017.
 814 Metaviromes of extracellular soil viruses along a Namib Desert aridity gradient.
 815 Genome Announc 5:4–5. doi:10.1128/genomeA.01470-16

816 Zablocki O, van Zyl L, Trindade M. 2018. Biogeography and taxonomic overview of
 817 terrestrial hot spring thermophilic phages. Extremophiles 22:827–837.
 818 doi:10.1007/s00792-018-1052-5

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Figures

Figure 1: Sample site and environmental conditions of the sampled soils. A) Map of the Namib Desert gravel plain location. Modified from European Space Agency, ESA/Envisat [CC BY-SA 3.0 IGO](https://creativecommons.org/licenses/by-sa/3.0/). B) View of a representative portion of the gravel plain sampling site. C) Environmental conditions over the experimental period: Air temperature, humidity and wind were recorded by the nearby Gobabeb meteorological station (Southern African Science Service Centre for Climate Change and Adaptive Land Management (SASSCAL), station 8893).

Figure 2: Principal components analysis of A) KEGG Orthologs (KO) annotated transcripts, and B) taxonomic (family) assignment of transcripts. The arrow designates the temporal transition pathway.

Figure 3: Ribosomal protein gene transcripts as a fraction of the total (Rp:T) among the most transcriptionally active microbial classes in the Namib soil community (>1% TPM average in dry or watered samples). Left panel shows *early* response classes whose Rp:T ratios peak within the first hour after watering. Right panel includes classes with Rp:T maxima 3 and 7 hours after watering (*middle* and *late* response taxa, respectively).

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842 **Figure 4:** Temporal transcription patterns of the 30 most variable genes among
843 those with significantly differential transcription along the time series. Transcript data
844 was aggregated along taxonomic (class) as well as functional (KEGG Orthologs) groups
845 for the analysis. Values were normalized using the Variance Stabilizing Transformation
846 (*DESeq* R package).

Figure 5: A) Nitrogen cycling and B) phosphate assimilation system transcription in the surface soil microbial community. Average TPM measurements are provided for the dry plot samples (orange arrows) and wetted samples up to 7 hours post watering (green arrows). Asterisks indicate significant differentially transcribed KOs at community level at any point along the time series. Panel B figure modified from León-Sobrinó *et al.*, 2019.

Figure 6. Temporal changes in viral protein gene transcripts as a fraction of the total (Vp:T ratios; %) for watered and dry (control) soil samples. The PW (Pre-watering) value represents the mean $\pm$ sd of Vp:T ratios in all control samples.

Figure 7. Network analysis, relating Water Experiment (WE) phage sequences to known viral sequences from the RefSeq Database. Circled clusters present viral contigs identified in this study. Shapes indicate major viral families, RefSeq sequences are in grey and WE contigs are colored (yellow and green for dry and watered samples, respectively). Each node is depicted as a different shape, representing viruses belonging to Myoviridae (triangle), Podoviridae (circle), Siphoviridae (diamond), or uncharacterized viruses (V shape). Single shapes represent viral singletons identified in this study. Edges (lines) between nodes indicate statistically weighted pairwise similarity scores (see Materials and Methods) of ≥ 1 .

Figure 8. Response model of microbial communities to water events in hyperarid desert soils.