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Nutrient acquisition, rather than stress response over diel cycles, drives microbial transcription in a dessicated Namib Desert soil

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Abstract

Hot desert surface soils are characterised by extremely low water activities for large parts of any annual cycle. It is widely assumed that microbial processes in such soils are very limited. Here we present the first metatranscriptomic survey of microbial community function in a low water activity hyperarid desert soil. Sequencing of total mRNA revealed a diverse and active community, dominated by *Actinobacteria*. Metatranscriptomic analysis of samples taken at different times over three days indicated that most functions did not fluctuate on a diel basis, except for a eukaryotic subpopulation which was induced during the cooler night hours. High levels of transcription of chemoautotrophic carbon fixation genes contrasted with limited expression of photosynthetic genes, indicating that chemoautotrophy is an important alternative to photosynthesis for carbon cycling in desiccated desert soils. Analysis of the transcriptional levels of key N-cycling genes provided strong evidence that soil nitrate was the dominant nitrogen input source. Transcriptional network analyses and taxon-resolved functional profiling suggested that nutrient acquisition processes, and not diurnal environmental variation, were the main drivers of community activity in hyperarid Namib Desert soil. While we also observed significant levels of expression of common stress response genes, these genes were not dominant hubs in the co-occurrence network.

Background

Arid lands (deserts) are defined as having a level of precipitation (P) below the potential evapotranspiration (PET) level (P/PET < 1). Such lands cover an estimated

one-third of Earth's terrestrial surface (Laity, 2008) and are projected to expand in current climate change scenarios (Reich *et al.*, 2001). The Namib Desert, located along the western coast of Namibia and extending into southern Angola and northern South Africa, is the oldest (ca. 5 million years) continuously hyperarid (P/PET < 0.05) desert on Earth (Seely and Pallet, 2008).

According to current models, aridity results in habitat fragmentation, both geographically leading to "islands" of microbial biomass and diversity and temporally, producing long periods of functional inactivity (Pointing and Belnap, 2012; Collins *et al.*, 2014). However, recent evidence suggests that some activity is retained under these extreme conditions (Gunnigle *et al.*, 2017; Schulze-Makuch *et al.*, 2018). In recent years, the microbial ecology of various Namib Desert edaphic niches has been extensively studied (e.g. Scola *et al.*, 2017; Johnson *et al.*, 2017; Ronca *et al.*, 2015; Frossard *et al.*, 2015).

RNA sequencing has been employed to study microbial community functional patterns in many different aquatic and terrestrial ecosystems. The short life-span and high turnover of messenger RNA (Belasco and Brawerman, 1993) allows ephemeral states of microbial communities to be captured without significant interference from legacy biomolecules or inactive microbial populations, as might be the case in DNA- or protein-based studies (Nielsen *et al.*, 2006). In desert environments, active community changes over diel cycles or after rainfall have been described by 16S rRNA amplicon transcriptome studies (Gunnigle *et al.*, 2017; Štovíček *et al.*, 2017).

In this study, we analyzed 12 shotgun metatranscriptomes from hyperarid desert soils sampled over the course of 3 days. The experiment was designed to assess the diel

55 transcriptional activity of desert edaphic microbial communities, particularly focusing
56 on nutrient acquisition and stress response mechanisms. We also aimed to identify
57 the key community members responsible for nutrient (C, N, P) cycling. Given the
58 fluctuations in light, temperature and humidity to which desert soil communities are
59 exposed within a daily cycle, we hypothesized that functional transcriptional profiles
60 would also show distinct diurnal cycles.

61 Results and Discussion

62 **Soil physicochemical characteristics.** Soils were collected from a calcrete gravel plain
63 site near the Gobabeb Research and Training Centre in the central Namib Desert
64 (23°33'34"S 15°02'25"E) (Scholz, 1972) after a prolonged dry period (Supplementary
65 Table S1) . We implemented a three day sampling strategy with soil collection at near
66 sunrise (6:00 h), at midday (12:00 h), at near sunset (18:00 h), and at midnight (24:00
67 h). Surface soil temperatures ranged from 21.4 ºC to 51.3 ºC, and soil air humidity
68 ranged from 13% to 27.7% (Supplementary Fig. S2). An average photosynthetically
69 active radiation (PAR) of 1722 ± 22 µmol photons m^{-2} s^{-1} was measured at 12:00 h, but
70 was negligible or zero at 6:00, 18:00, and 24:00 h. Consistent soil respiration was
71 recorded throughout the experiment (Supplementary Table S2).

72 The physicochemistry of soil samples was globally homogeneous. Localized
73 heterogeneity was observed in four quadrats and related mostly to salt or phosphate
74 concentration (Supplementary Table S2, Fig. S3).

75 **Library construction and sequence data.** A sample from each time point was selected
76 for library construction (n=12) based on homogenous soil physicochemical

77 characteristics. Sequencing of cDNA libraries produced 285 million reads in total, with
78 an average read length of 76 nt. After quality filtering and discarding rRNA and human-
79 derived reads, 268 million high-quality reads were retained (Table S3).

80 **Taxonomic composition of the active soil microbial community.** The taxonomic
81 composition of the active microbial populations was similar throughout the study
82 period (Supplementary Fig. S4), in spite of observed variations in temperature,
83 humidity and light (Supplementary Fig. S2, Table S2). A single exception was the Day 2,
84 24:00 h library, which contained an unusually large proportion of fungal (19.5%,
85 compared to an average of 2.8% in the remaining libraries) and *Firmicutes* (38.5%,
86 compared to 5.9%) phylotypic sequences (Supplementary Fig. S4). This result most
87 likely represented a random biological outlier, and this library was excluded from
88 further analyses.

89 The phylogenetic analysis of the remaining 11 libraries showed that members of the
90 domain Bacteria were most active (94.2 ± 2.6% of transcripts), with Eukarya
91 comprising 4.3 ± 1.8% and Archaea 1.5 ± 1.0%. Virus-classified reads amounted to 0.04
92 ± 0.02% (Fig. 1A). Despite representing only a minor proportion, this is, to our
93 knowledge, the first report of transcriptionally active viruses in dessicated hot desert
94 soils, where lysogeny is considered to be the dominant state of virus populations
95 (Zablocki *et al.*, 2015). However, the read volume was insufficient to provide a
96 comprehensive survey of transcribed viral genes.

97 Seven bacterial phyla (*Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*,
98 *Chloroflexi*, *Cyanobacteria*, and *Deinococcus-Thermus*) and one eukaryal phylum
99 (*Ascomycota*) each contributed more than 1% of the classified reads, jointly comprising

93.1 ± 2.3% of the total active community (Fig. 1A and Supplementary Fig. S4). The most active phylum globally was *Actinobacteria*, producing 52.1 ± 5.4% of the classified transcripts, followed by *Proteobacteria*, with 20.2 ± 2.6% of the transcripts. Two prominent actinobacterial families, *Geodermatophilaceae* and *Rubrobacteraceae*, represented 8.2 ± 1.8% and 7.0 ± 1.9%, respectively (Fig. 1A). Both families have been routinely detected in desert soils and their members typically exhibit high stress tolerance and are metabolically versatile (Rainey *et al.*, 2005; Favet *et al.*, 2013; Sghaier *et al.*, 2016; Albuquerque and da Costa, 2014; Normand *et al.*, 2015).

Functional profile of the microbial community. All core metabolic pathways were transcribed (Fig. 1B), including replication genes, indicating that the active fraction of the soil microbial community had complete functionality.

This observation implies the existence of a xeroresistant microbial community in this hyperarid desert niche. Tolerance; i.e., survival with impaired or no activity and no growth, is regarded as the most common strategy adopted by microbial communities under extreme xeric stress, such as in hyperarid desert soils (Lebre *et al.*, 2017). Hyperaridity results in habitat fragmentation and concentrates activity in sheltered "islands of fertility" and during brief wet periods (Pointing and Belnap, 2012; Collins *et al.*, 2014). Active microbial populations have recently been detected in hyperarid soils from the Atacama Desert (Schulze-Makuch *et al.*, 2018), although at very reduced activity levels that suggest a temporally or metabolically limited state. However, our transcription results, which demonstrate the presence of a functional fraction of the microbial community, suggest that resistance, rather than tolerance, is a strategy adopted by some of the resident taxa. Resistance is here defined as the maintenance

of function, despite the impositions of extreme environmental parameters (i.e., hyperaridity) (Harrison *et al.*, 2007).

The coexistence of dessication-resistant and -tolerant microbial taxa has been observed in non-arid soils, where Actinobacteria remain active during dry periods whereas Acidobacteria become the dominant active group upon rewetting (Barnard *et al.*, 2013). Our findings therefore extend this dual-response model to soils under extreme xeric stress.

Genes encoding elements of stress resistance and damage repair mechanisms were highly transcribed. Chaperone genes *groEL* and *dnaK* (4.9% and 1.5% of the classified transcripts, respectively) (Fig. 1B), and protease genes involved in protein quality control (e.g., *clpX/P* and *lon*; 1.7% and 0.7%, respectively) were among the most transcribed. Furthermore, the high relative abundances of peroxisomal orthologs (2.4%), such as superoxide dismutase (SOD) and catalase (*katE*), as well as DNA repair gene transcripts (*recA*, *uvr*, Fig. 1B), support the widely held view that radiation- and desiccation-induced damage (particularly related to oxidation processes) are the major stresses for microbial cells in hyperarid hot desert soils (Makhalanyane *et al.*, 2015). The production of compatible solutes and capsule formation, which are common microbial adaptation mechanisms for desiccation tolerance (Lebre *et al.*, 2017), were suggested by polysaccharide and trehalose biosynthesis gene transcripts such as the alpha-glucan branching enzyme gene *glgB* (0.4% of transcripts) (Rashid *et al.*, 2016) (Fig. 1B). Overall, the transcriptional profile of the microbial community coherently reflects known strategies of desiccation resistance predicted from genomic analyses of

145 desiccation-tolerant microorganisms (Lebre *et al.*, 2017; Schulze-Makuch *et al.*, 2018),
146 allowing full activity of a subpopulation throughout hyperarid periods.

147 **Nutrient cycling and key active taxa.** Carbon, nitrogen, and phosphorus are the major
148 limiting nutrients for microbial communities, and for oligotrophic desert soil
149 communities in particular (Cleveland and Liptzin, 2007; Delgado-Baquerizo *et al.*, 2013;
150 Johnson *et al.*, 2017). The transcriptional activity of orthologs involved in assimilation
151 pathways of these nutrients was thus investigated, to evaluate the contributions of
152 specific taxa to these processes in the community.

153 High levels of functional redundancy were evident (Fig. 2). However, some important
154 ecosystem functions appeared to be taxon-specific. For example, nitrate reductase
155 (*nar*) genes, which encode key enzymes in nitrogen assimilation in soils (Merrick and
156 Edwards, 1995) (Fig. 3A), were transcribed almost exclusively by members of the
157 *Nitrospiraceae* family (Fig. 2A), indicating that this family plays a key role in the
158 nitrogen cycling of Namib desert soil communities.

159 **Nitrogen assimilation.** Nitrogen-fixing bacterial taxa such as *Geodermatophilaceae*,
160 *Frankiaceae* and *Rhizobiales* (Merrick and Edwards, 1995; Sellstedt and Richau, 2013)
161 were among the most active taxa (Fig. 1A). However, transcripts relating to the
162 nitrogen metabolism KEGG pathway represented a small portion (0.2%) of our soil
163 metatranscriptomes and virtually no *nifD* nitrogenase transcripts were detected (Fig.
164 3A). These findings are compatible with recent observations that hypolithic
165 communities, and not surface soil communities, were the primary sources of N_2-
166 fixation in Namib Desert gravel plains (Ramond *et al.*, 2018).

Transcriptome data suggested that nitrate reduction, most transcribed by the *Nitrospiraceae* family, and nitrite reduction primarily transcribed in actinobacterial taxa (*nar* and *nir* genes, respectively, Fig. 2A) were the dominant processes in the generation of biologically available nitrogen in the community from a NO_3^- and NO_2^- reservoir (Fig. 3A). These nitrogen species may be accumulated in soils during infrequent wet periods, possibly as a result of the activation of genes and microorganisms inhibited during dessicated conditions (Scherer *et al.*, 1984), or from dry atmospheric deposition processes (Báez *et al.*, 2007; Jia *et al.*, 2016).

Phosphorus and Sulfur assimilation. Most phosphorus is available to soil microbial communities as inorganic phosphate (Pi), solubilized from the mineral soil fraction or released from organic molecules by the alkaline phosphatase (White and Metcalf, 2007). *Pst* phosphate transporter gene transcripts were abundant in the community (1390 average counts per million, cpm). Organic phosphate sources were also possibly exploited, as suggested by transcription of the *phn* phosphonate transporter gene (153 cpm) and especially the sn-glycerol 3-phosphate (G3P) transporter gene *ugp* (1228 cpm) (Fig. 3B). Although the expression of *phn* and *ugp* can be inhibited by Pi (Schowanek and Verstraete, 1990; Brzoska *et al.*, 1994), organic P utilization may still be an important microbial community trait in oligotrophic desert environments (Vikram *et al.*, 2016). The *ugp* genes were principally transcribed by members of the Order *Rhizobiales* (Class *alpha-Proteobacteria*), potentially replacing Pi transport as a phosphorus acquisition mechanism (Fig. 2B). Plant exudates or membrane phospholipids are possible sources of G3P in soils (Collins *et al.*, 2014; Lidbury *et al.*, 2017; Ding *et al.*, 2012). The *glpQ* gene product can cleave these compounds, releasing G3P and triggering activation of the *ugp* transporter genes (Brzoska and Boos, 1988).

191 *GlpQ* is an extracellular enzyme which has been implicated in cooperative interactions
192 between proteobacteria (Lidbury *et al.*, 2017). In our dataset, *glpQ* was mostly
193 transcribed in *Actinobacteria*. The most active actinobacterial family
194 *Geodermatophilaceae*, however, transcribed *glpQ*, but not the G3P transporter *glp* or
195 the alkaline phosphatase *pho* genes, which would dephosphorylate G3P, releasing Pi
196 for its own consumption (Fig. 2B). Our data therefore suggest a putative interaction
197 between *Geodermatophilaceae* and *Rhizobiales*, with the former providing access to
198 phosphorus as G3P for the latter. This interaction may be of considerable importance
199 for desert community maintenance, as both taxa were amongst the most
200 transcriptionally active (8.2% and 6.1%, respectively) (Fig. 1A).

201 Reductive sulfate assimilation (*cys* genes) was the dominant transcribed S-cycling
202 pathway in the community. However, transcripts for the sulfate transporter *cysPUWA,*
203 were mostly associated to proteobacteria, particularly the Burkholderiales family,
204 suggesting a central role of this group in sulfur assimilation and cycling.

205 **Carbon fixation.** A defining feature of arid soils is low productivity and a low organic
206 carbon content (Delgado-Baquerizo *et al.*, 2013). Hyperaridity imposes severe
207 constraints on oxygenic photosynthesis, for which water is the electron donor
208 (Warren-Rhodes *et al.*, 2006). Furthermore, soil communities outside of sheltered
209 fertile islands (i.e., hypoliths, endoliths, or biological soil crusts) typically have a very
210 low abundance of the phototrophic cyanobacteria (Stomeo *et al.*, 2013; Makhalanyane
211 *et al.*, 2013). Perhaps not surprisingly, transcription of photosynthetic pathway genes
212 and phototrophic organisms was limited in our dataset (Fig. 1B, Fig. 1A). Notably, reads
213 classified within the Glyoxylate and Dicarboxylate pathway (2.0%) exceeded those

assigned to photosynthetic KEGG pathways and, surprisingly, also significantly exceeded those from the TCA Cycle (0.3% and 1.6%, respectively, two-tailed t-test $p < 0.005$) (Fig. 1B). We also observed a higher number of transcripts assigned to acetyl-CoA synthetase (ACSS, 9874 cpm) and formate dehydrogenase (FDH, 10254 cpm) compared to RuBisCO (*rbcL/S*, 2672 cpm) (Fig. 1B). These observations strongly suggest that chemoautotrophic ("dark") carbon fixation and/or CO_2 reassimilation mechanisms are important microbial processes by which inorganic C enters the soil microbial community. Chemoautotrophic carbon fixation has been shown as an important process in marine environments, even when photosynthesis is active (Palovaara *et al.*, 2014; Aylward *et al.*, 2015), and is a potentially major process in soils (King and Weber, 2007; Pratscher *et al.*, 2011). Therefore, we examined the activity of carboxylase genes in greater depth, and their distribution between different families of microorganisms.

Although RuBisCO gene transcripts (*rbc*) from the Calvin-Benson-Bassham (CBB) cycle were significant (average 2672 cpm) (Fig. 1B), the majority were assigned to non-photosynthesizing *alpha-Proteobacteria* rather than to *Cyanobacteria* (Fig. 2C). This suggested that the CBB cycle acted predominantly in chemoautotrophic CO_2 fixation or as an electron sink (Badger and Bek, 2008; McKinlay and Harwood, 2010), rather than in photosynthesis.

Orthologs of the acetyl-CoA synthase ACSS (9874 cpm), CO dehydrogenase *coxS* (1701 cpm) and formate dehydrogenase FDH (10254 cpm) genes, involved in the reductive acetyl-CoA cycle (Wood-Ljungdahl pathway), were significantly transcribed in a wide range of taxa (Fig. 2C). These carboxylases are widely distributed in soil bacteria (King and Weber, 2007) and are active in desert actinobacteria (Sghaier *et al.*, 2016). The

activity of these genes in Namib Desert soil microbial communities may be related to the very low energy requirements and the capacity to coassimilate one-carbon compounds or acetate of this pathway (Fuchs, 2011), making it well suited to oligotrophic niches.

The reductive citric acid cycle (Arnon-Buchanan cycle) carboxylases *kor* (2-oxoglutarate synthase) and *icd* (isocitrate dehydrogenase) were transcribed by many actinobacterial families, but surprisingly not by *Rubrobacteraceae* (Fig. 2C). Instead, *Rubrobacteraceae* transcribed the acetyl and propionyl-CoA carboxylase *pccB* and *accA/C/D*, the phosphoenolpyruvate (PEP) carboxylase *pckA* and the pyruvate synthase *por*, which participate in other pathways of chemoautotrophic carbon fixation (Fuchs, 2011) (Fig. 2C). These pathways could only be partialy detected in *Rubrobacteraceae*, as several key genes (e.g., malonyl-CoA reductase, 4-hydroxybutyryl-CoA dehydratase) were not identified. These pathways, either full or partial, allow prokaryotes to coassimilate reduced and uncommon C compounds and to fix carbonate (Fuchs, 2011; Zarzycki and Fuchs, 2011). Our results therefore suggest that the actinobacterial *Rubrobacteraceae* family may be important in inorganic carbon acquisition in desert soils, partly due to a high plasticity in chemoautotrophic metabolism.

Circadian differential gene expression. Environmental variations often cause microbial communities to exhibit differential activity profiles over temporal timescales (e.g., daily or seasonally), both in phototrophic and non-phototrophic groups (Klatt *et al.*, 2013; van der Meer *et al.*, 2005; Ottesen *et al.*, 2013, 2014; Aylward *et al.*, 2015).

Diel transcriptional periodicity was examined using EdgeR (Robinson *et al.*, 2010). Time pairs were contrasted independently, as well as "day" (12:00 and 18:00) versus "night"

(24:00 and 6:00) groups as defined by their contrasted temperature and humidity records (Supplementary Fig. S2). Interestingly, pairwise comparisons identified no differentially expressed orthologs ($p > 0.05$) between the 12:00 and 18:00 or between the 24:00 and 6:00 datasets. When "day" and "night" data were contrasted, 13 of 2265 orthologs (0.57%) were significantly ($p < 0.05$) induced during the night (Supplementary Table S4). None were highly transcribed orthologs, suggesting that under extreme dry conditions, desert soil communities are generally functionally stable and that their principal functions are not regulated on a diel scale. This conclusion has implications in terms of the perceived drivers of microbial community function, as our results suggest that the constant xeric stress is a more significant driver of *in situ* functionality than daily environmental variations (temperature, air moisture or light). We predict that this functional stability would only be substantially disrupted by stochastic events such as rainfall, which is recognised as a main driver of community assembly and activity in arid soil environments (Belnap *et al.*, 2005; Pointing and Belnap, 2012; Frossard *et al.*, 2015; Scola *et al.*, 2017).

Surprisingly, we observed a marked enrichment in differentially transcribed eukaryal orthologs, including tubulin, dynein, myosin, SF3B, *dnaJ* and ANP1 genes (Supplementary Table S4). This suggested that the active fungi, which only represented 2.8% of the total transcripts, were most active during the cooler and higher atmospheric humidity night hours, contrary to the generally stable activity pattern observed for the rest of the community. This is consistent with observations made on fungi and lichens from arid environments, which appear to grow optimally during small air moisture pulses (Palmer *et al.*, 1987; Jacobson *et al.*, 2015).

283 **Transcriptional network analysis.** The temporal co-variation of KOs was determined in
284 order to examine whether coordinated patterns of gene transcription existed within
285 the soil community. 624 orthologs were used to construct a transcriptional network,
286 83.3% of which (520) clustered into 4 distinct modules (A to D, Fig. 4). The larger
287 clusters A and B were composed of positively interrelated orthologs, although no
288 specific functional enrichment within each module was observed.

289 The principal transcriptional network clusters were connected through three
290 orthologs: the dihydrolipoamide dehydrogenase DLD, the G3P transporter subunit
291 *ugpB* and the glutamate dehydrogenase gene *gudB* genes. These genes occupy
292 network hub positions and therefore changes in their transcriptional status could
293 result in large shifts in community function. The dominant metabolic function
294 associated with the highly transcribed (3917 cpm, Fig. 1B) DLD gene is in the TCA cycle,
295 but has also been shown to affect sugar transport and capsule formation via direct
296 interactions with membrane transporters (Tyx *et al.*, 2011). The importance of
297 exopolysaccharides in desiccation resistance (Lebre *et al.*, 2017), and the TCA cycle in
298 carbon metabolism regulation, support the centrality of DLD in the community
299 network. The gene *ugpB*, as previously discussed, was linked to the rhizobial
300 community as part of a nearly exclusive phosphorus assimilation mechanism (Fig. 2B).
301 The *gudB* gene product catalyzes the synthesis of glutamate, the principal acceptor
302 metabolite in NH_3 assimilation (Merrick and Edwards, 1995)(Fig. 3A).

303 The network was also characterized by a group of orthologs connecting the main
304 clusters A and B. Three of these are involved in nitrogen (*nirA*) (Merrick and Edwards,

1995) (Fig. 3A), sulfur (*cysD*) (Pinto *et al.*, 2004) and central carbon metabolism (*scoB*) (Corthésy-Theulaz *et al.*, 1997) (Fig. 1B).

Globally, network analysis revealed that transcriptional activity of the community is structured around a selection of hub genes involved in central steps of nitrogen (*nir*A, *gudB*) (Fig. 3A) and sulfate assimilation (*cysD*, *cysN/C*), phosphorus acquisition (*ugpB*) (Fig. 3B) and carbohydrate metabolism (DLD, *scoB*), rather than around genes related to environmental stress resistance and damage repair (e.g. chaperones, proteases, SOD, *uvr*, *rec*), despite these genes being consistently active (Fig. 1).

Conclusions

It is widely accepted that the extreme conditions in hot desert open soils limit both microbial and plant life (Pointing and Belnap, 2012; Makhalanyane *et al.*, 2015), and that microbial activity is spatially fragmented, temporally limited and water-driven (Pointing and Belnap, 2012; Belnap *et al.*, 2005; Collins *et al.*, 2014). We here show that there is a diverse and consistently active edaphic microbial community in open Namib Desert desiccated soils, dominated by non-photosynthetic bacteria, with a substantial actinobacterial and rhizobial component. Transcripts from all central metabolic pathway genes were detected, suggesting consistent metabolic activity during the study period. We therefore suggest that resistant microbial subpopulations remain active throughout long dry periods, rather than surviving in inactive states. Despite the observation of regular environmental fluctuations, no major diel changes were observed in prokaryotic activity, although a significant activation of fungal genes was noted during the night hours.

We identified the key microbial taxa responsible for the variety of strategies for carbon, nitrogen and phosphorus acquisition in the Namib Desert soil. Namely *Nitrospiraceae* (nitrate reduction), *Actinobacteria* (nitrite reduction, carbon fixation) and *alpha-Proteobacteria* (glycerol 3-phosphate assimilation) appeared as key functional members of the soil community.

Transcriptional network analysis of the community revealed a group of genes, involved in carbon, nitrogen, phosphorus and sulfur metabolism, in hub positions. This result supports our contention that the main driver of community functionality in Namib gravel plain soil under hyperarid conditions is nutrient assimilation rather than either environmental changes linked to day-night fluctuations (temperature, light, humidity) or the activity of stress resistance and repair genes.

Chemoautotrophic carbon fixation genes were among the most transcribed overall, indicating that this constitutes an important form of carbon assimilation under conditions where photosynthesis is restricted. We hypothesize that non-photosynthetic carbon fixation could be a strong adaptive factor in hyperarid, carbon-poor soils. Biochemical evidence is required to quantify the contribution of chemoautotrophy to the microbial community carbon flux.

Most notably, microbial N acquisition appeared to be limited to dissimilatory nitrate reduction. While some of the most transcriptionally active actinobacterial and rhizobial taxa are known to possess the capacity for dinitrogen fixation, there was no evidence that this was a significant contribution to nitrogen input budgets under desiccated soil conditions. We suggest that dinitrogen fixation processes may only become significant in times of higher water activity (such as after rainfall).

Materials and Methods

Sampling procedure

The sampling site was located in the gravel plains of the central Namib Desert (23°33'34"S 15°02'25"E), Namibia, approximately 56 km from the coast. The mean annual precipitation at the site is estimated at 25 mm, principally derived from nocturnal marine fog (Eckardt *et al.*, 2013). A 10 x 10 m experimental plot was sub-divided into 64 quadrats (Supplementary Fig. S1). Surface soils (0-4 cm) were collected at 6 hourly intervals (6:00, 12:00, 18:00, and 24:00 h) over three days from the 12th to the 14th April 2016. Two Hygrochron iButton sensors (Embedded Data Systems, Lawrenceburg KY, USA) were positioned at the corners of the plot, at ~2 cm depth, recording temperature and relative humidity at 4 minute intervals for the length of the experiment (Fig. S1). Soil respiration measurements were performed at the designated sampling times at four points within the plot using a LI-8100 IRGA (LI-COR Biosciences, Lincoln NE, USA), covering an area of 83.7 cm^2 with a 3 litre chamber for 30 seconds (Supplementary Fig. S1). Photosynthetically active radiation (PAR) was measured using a photometric sensor (Quantum, LI-COR) at the same internal plot locations. Surface soil samples (0-4 cm) were collected at 6 hourly intervals (6:00, 12:00, 18:00, and 24:00 h) over three days from the 12th to the 14th April 2016. Three randomly selected quadrats were sampled at each time point (Supplementary Fig. S1). 20 g soil samples were immediately preserved on-site in RNAlater solution (Sigma-Aldrich, St. Louis MO, USA), temporarily stored at -20ºC at the Gobabeb Research and Training Center and during transport to the laboratory, and subsequently at -80ºC prior to total RNA extraction. An additional 400 g of soil for physicochemical analysis was collected in

373 WhirlPak bags (Nasco, Fort Atkinson WI, USA) and preserved at 4ºC before
374 physicochemical analyses. Soil pH, conductivity, cation exchange capacity (CEC), total
375 nitrogen (%N), phosphorus (P), sodium (Na), potassium (K), calcium (Ca), magnesium
376 (Mg), Chloride (Cl), Sulphate (SO_4), ammonium (NH_4) and nitrate (NO_3) contents were
377 analyzed by Bemlab (Pty) Ltd. (http://www.bemlab.co.za/; Strand, Western Cape,
378 South Africa) using standard protocols.

379 *Total RNA purification*

380 Soils from twelve physicochemically representative quadrats representing all sampling
381 times were selected for RNA extraction (Supplementary Fig. S1, Table S2). Frozen,
382 RNAlater-preserved soils were thawed at 4ºC, centrifuged at 14,500 rpm for 5 minutes
383 and supernatants were discarded. 5 volumes of ice-cold 10 mM Tris-HCl 1 mM EDTA
384 pH 6.5 buffer containing 100 mM NaH_2PO_4 were added to the soil to remove RNAlater
385 salts. The supernatant was discarded after rapid (4 min) centrifugation at 4ºC. 0.5
386 volumes lysis buffer (5% CTAB, 0.7 M NaCl, 240 mM KH_2PO_4, pH 8) and an equal
387 volume of TRI Reagent (Sigma-Aldrich) were added, and samples were vortexed at high
388 speed for 30 seconds. RNA purification proceeded according to the manufacturer's
389 instructions. Extracted and purified total RNA was incubated with DNAseI (Invitrogen,
390 Carlsbad, USA) and precipitated in the presence of 20% isopropanol and 15 ng
391 glycogen co-precipitant (GlycoBlue, Invitrogen). RNA concentration and integrity were
392 analysed using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham,
393 USA) and 1% agarose gel electrophoresis. The absence of RT-PCR inhibitors was tested
394 using the Transcriptor cDNA Synthesis Kit v9 (Roche, Indianapolis IN, USA) and
395 universal bacterial 16S rRNA gene primers E9F (5'-GAGTTTGATCCTGGCTCAG-3') and

U1510R (5'-GGTTACCTTGTTACGACTT-3') (Reysenbach and Pace, 1995; Hansen *et al.*, 1998).

Library construction and sequencing

1 µg DNA-free total RNA from each sample was used for each sequencing library. Due to low RNA yields, we combined RNA from two Day 2 6:00h quadrats (Supplementary Table S1). Construction of rRNA-depleted libraries was carried out with the ScriptSeq Complete Gold Kit (Epidemiology) (Epicentre, Madison WI, USA), following the manufacturer's instructions. Briefly, rRNA was removed by hybridization with bead-immobilized prokaryotic and eukaryotic 28S, 23S, 18S, 16S, 5.8S, 5S, mt16S and mt12S probes prior to RNA fragmentation and reverse transcription with tagged random hexamer primers. cDNA was amplified with TruSeq adaptors containing unique indexes (ScriptSeq Primer Set 1, Epicentre) for 15 PCR cycles. Libraries were purified using AMPure XP beads (Beckman-Coulter, Brea, USA) and final yields were measured with the High Sensitivity dsDNA reagents on a Qubit 2.0 fluorometer (Invitrogen). Multiplexed samples were quality and size analyzed in a High Sensitivity D1000 TapeStation (Agilent, Waldbronn, Germany). Libraries were single-end sequenced in a NextSeq500 v2 platform using the NextSeq 500/550 High Output v2 kit (Illumina, San Diego, USA). RNA-seq data were deposited in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) and can be accessed using the reference E-MTAB-6601.

Read quality trimming was performed using Prinseq-lite v0.20.4 (Schmieder and Edwards, 2011) on both read ends with a mean Phred value of ≥30 in a 6 base sliding window. Reads shorter than 40 bases after trimming were discarded. rRNA and human-derived reads were removed from the dataset using Bowtie2 (Langmead and

419 Salzberg, 2012) with a database of large- and small ribosomal subunit genes from
420 SILVA (https://www.arb-silva.de/), 5S rRNA genes from the 5SRNAdb repository
421 (Szymanski *et al.*, 2016) and the GRCh38 human genome primary assembly
422 (ftp://ftp.ncbi.nlm.nih.gov/genomes/archive/old_genbank/Eukaryotes/vertebrates_m
423 ammals/Homo_sapiens/GRCh38/seqs_for_alignment_pipelines/GCA_000001405.15_G
424 RCh38_no_alt_analysis_set.fna.bowtie_index.tar.gz).

425 *Analysis of sequencing reads*

426 Functional and taxonomic profiling, and differential transcription analyses were
427 performed using R version 3.3.3 (R Core Team, 2017). Read taxonomy was inferred
428 from the NCBI Referece Sequence (RefSeq) database, and function was assigned based
429 on the Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthologs (KO) database
430 (Kanehisa *et al.*, 2016) using the MG-RAST server (http://metagenomics.anl.gov/)
431 (Meyer *et al.*, 2008). Read count tables were assembled and analysed for temporal
432 expression changes using the EdgeR package (Robinson *et al.*, 2010) for all genes with
433 > 1 count per million (cpm) in at least 3 libraries (n=11). Normalized KEGG ortholog
434 counts were fitted to a generalized log-linear model (*glmQLFit* function) (Robinson and
435 Oshlack, 2010; McCarthy *et al.*, 2012; Lun *et al.*, 2016), and pairwise comparisons
436 between all time points were performed. Additionally, grouped "day" samples from
437 12:00 and 18:00 h were compared to "night" samples from 24:00 and 6:00 h. KOs were
438 considered significantly differentially expressed between time points below a false
439 discovery rate (FDR) corrected p-value threshold of 0.05.

440 Orthologs with average $\log_2$CPM values > 7 were used to construct a transcriptional
441 network (Fig. 5), excluding KEGG categories Human Diseases and Organismal Systems.

This threshold was selected as being below the common dispersion value calculated during differential expression analysis in order to reduce interference from high-variance, low-abundance transcripts. A transcriptional network was constructed using MENA's (Deng *et al.*, 2012) RMT-based modeling with a correlation cutoff of 0.900 ($p \leq 0.005$). Co-transcription was determined using Pearson's correlation coefficients across libraries (n=11). The network was visualized using Cytoscape v. 3.5.1 (Shannon *et al.*, 2003).

Figures

Fig. 1: (A) Average transcriptional activity of the 20 most transcriptionally active microbial phyla and families. Phyla are sorted according to their average transcription levels (*aveLogCPM* function). Family transcript abundance is given in average $\log_2$ counts per million (logCPM). Geod.: *Geodermatophilaceae*; Rub.: *Rubrobacteriaceae*; Methy.: *Methylobacteriaceae*; Rho.: *Rhodobacteraceae*; Brady.: *Bradyrhizobiaceae*; Bac.: *Bacillaceae*; Chroo.: *Chroococcales*; Nostoc.: *Nostocaceae*; Nitrosop.: *Nitrosopumilaceae*; Halobac.: *Halobacteriaceae*. **(**B) Average transcript abundance of KEGG orthologs in the 40 most transcriptionally active KEGG pathways. Pathways are sorted according to their average $\log_2$ counts per million (*aveLogCPM* function). Upper KEGG classes are highlighted on the left axis by color: Genetic Information Processing (red), Metabolism (blue) and Environmental Information Processing (green). Categories Human Diseases and Organismal Systems were not included in the plot. Orthologs of particular interest are named, in order of transcript abundance, besides their respective pathway. Abbreviations: ACSL: acyl-CoA synthetase; ACSS: acetyl-CoA synthetase; clpX: Clp protease ATP-binding subunit; *coxS*: carbon-monoxide dehydrogenase small subunit; *cysD*: sulfate adenylyltransferase subunit 2; *cysC*: adenylylsulfate kinase; cysN/C: bifunctional enzyme CysN/CysC; DLD: dihydrolipoamide dehydrogenase; *dnaK*: molecular chaperone DnaK; DPO1/3: DNA polymerase I/III; *dppF*: dipeptide transport system ATP-binding protein; FDHa/b: formate dehydrogenase alpha/beta subunit; *fdhA*: formaldehyde dehydrogenase; *glgB*: 1,4-alpha-glucan branching enzyme; *gltB*: glutamate synthase; *groEL*: chaperonin GroEL; *icd*: isocitrate dehydrogenase; *katE*: catalase; *lon*: Lon protease; *pckA*:

472 phosphoenolpyruvate carboxykinase; *rbcL*: ribulose-bisphosphate carboxylase large
473 chain; *recA*: recombination protein RecA; SOD2: superoxide dismutase; *ssb*: single-
474 strand DNA-binding protein; *treS*: maltose alpha-D-glucosyltransferase / alpha-
475 amylase; *uvrA*/*B*: excinuclease ABC; *xylA*: xylose isomerase.

476 **Fig. 2**: Correlation between family-level taxonomy and key KEGG ortholog transcription
477 from nitrogen (A), phosphorus (B) and carbon (C) assimilation pathways in selected
478 prokaryotic taxa. (A) Nitrogen metabolism orthologs: *gln*: glutamine synthetase; *glt*:
479 glutamate synthase; *gud*: glutamate dehydrogenase; *nar*: nitrate reductase; *nir*: nitrite
480 reductase. (B) Phosphorus assimilation and G3P metabolism orthologs: *glpD*: glycerol
481 3-phosphate dehydrogenase; *glpK*: glycerol kinase; *glpQ*: glycerophosphodiester
482 phosphodiesterase; *phn*: phosphonate transport; *phoABD*: alkaline phosphatase; *pst*:
483 phosphate transport; *ugp*: sn-glycerol-3-phosphate transport. (C) Carboxylase gene
484 orthologs involved in carbon fixation pathways: ACSS: acetyl-CoA synthase; *acc*: acetyl-
485 CoA carboxylase; FDH: formate dehydrogenase; *icd*: isocitrate dehydrogenase; *kor*: 2-
486 oxoglutarate synthase; *cox*: CO dehydrogenase; *pcc*: acetyl/propionyl-CoA carboxylase;
487 *pck*: PEP carboxylase; *por*: pyruvate synthase; *rbc*: RuBisCO. Phylum abbreviations: A :
488 *Actinobacteria*; B: *Bacteroidetes*; C: *Cyanobacteria*; Chf: *Chloroflexi*; F: *Firmicutes*;
489 Nitro: *Nitrospirae*; P: *Proteobacteria* (a: alpha, b: beta, d: delta, g: gamma). Only
490 families with > 6 average $\log_2$CPM for the selected genes were included in A and B.
491 Hierarchical clustering of rows and columns was performed with *hclust* function.

492 **Fig. 3**: Community-level transcription of nitrogen (A) and phosphorus (B) assimilation
493 pathways, including glycerol phosphate metabolism. Gene codes in bold highlight the
494 most abundant orthologs from a group performing the same function, when there is a

large transcript abundance difference. Numbers below gene codes show the total average counts per million (cpm) of all orthologs. Arrow thickness in each figure is proportional to the indicated cpm value.

Fig. 4: KEGG ortholog transcriptional network. Only orthologs with average abundances of $\log_2$ cpm > 7 in the complete dataset were used for computation. Node size is proportional to betweenness centrality and edge thickness is proportional to betweenness. Green or red edge lines indicate a shared positive or negative correlation, respectively. Ortholog abbreviations: ADK: adenosine kinase; *cysD*: sulfate adenylyltransferase (sulfate-activating complex); *cysN/C*: bifunctional enzyme *CysN/CysC* (sulfate-activating complex); DLD: dihydrolipoamide dehydrogenase; *gudB*: glutamate dehydrogenase; *ndk*: nucleoside-diphosphate kinase; *nirA*: ferredoxin-nitrite reductase; *pdhC*: pyruvate dehydrogenase; scoB: 3-oxoacid CoA-transferase; *ugpB*: sn-glycerol 3-phosphate transport system.

Declarations

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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-6601).

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. G. M.-K. provided logistical support and field advice in the Namib Desert. C. L.-S. performed all experimental work and bioinformatic analysis of the sequencing output. C. L.-S., J.-B. R. and D. A. C. participated in the interpretation of results and writing of the manuscript. D. A. C. provided funding.

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Figure 1

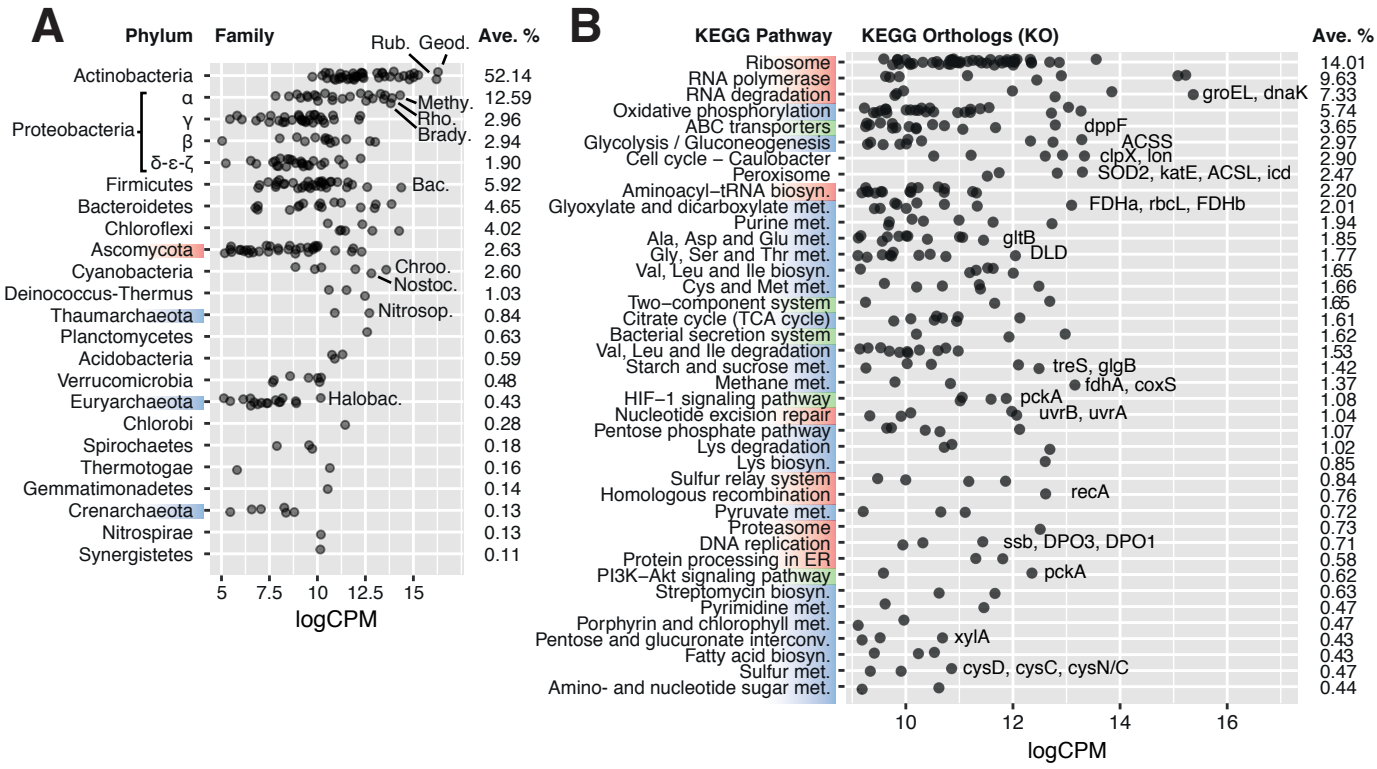

Figure 2

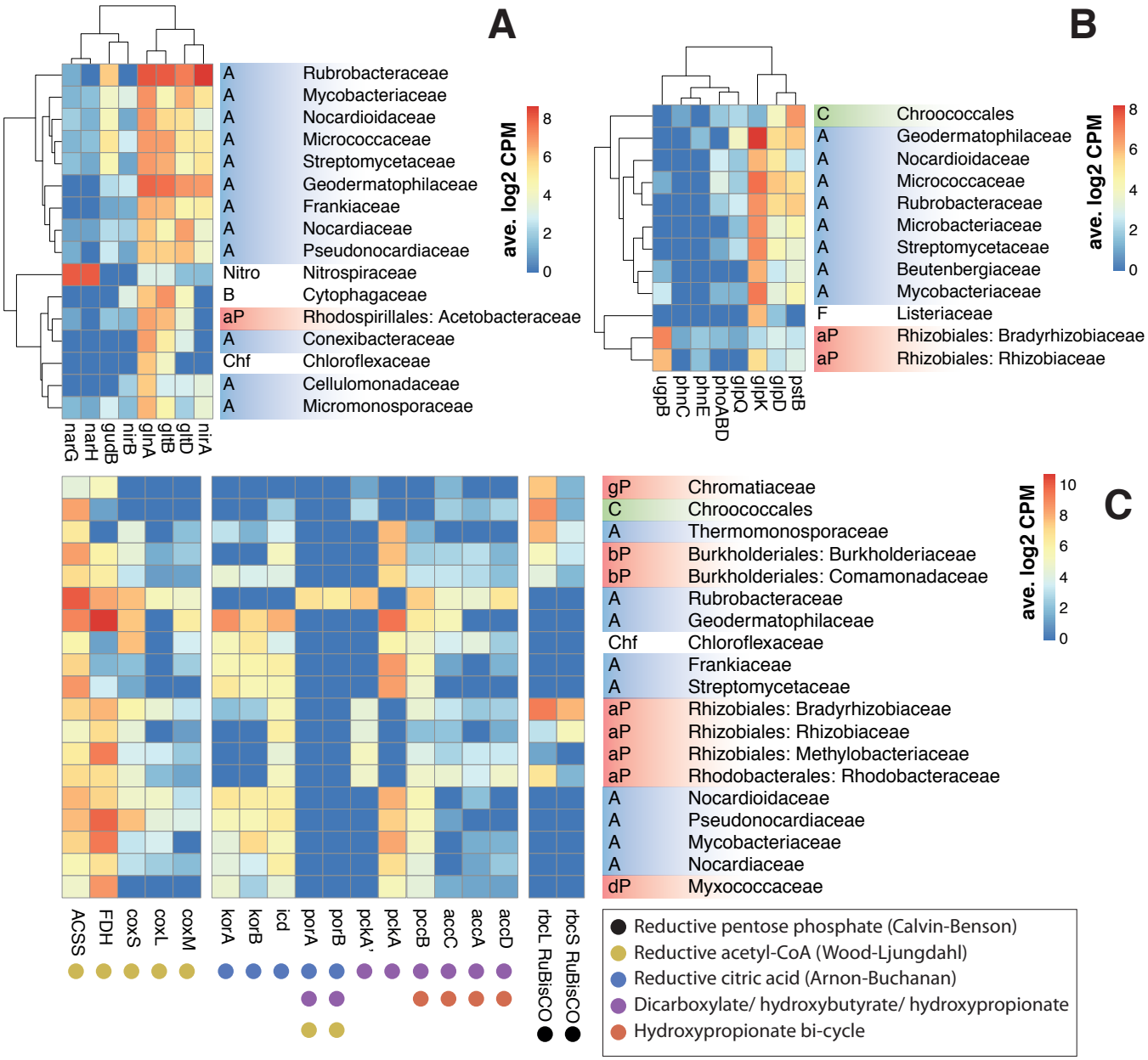

Figure 3

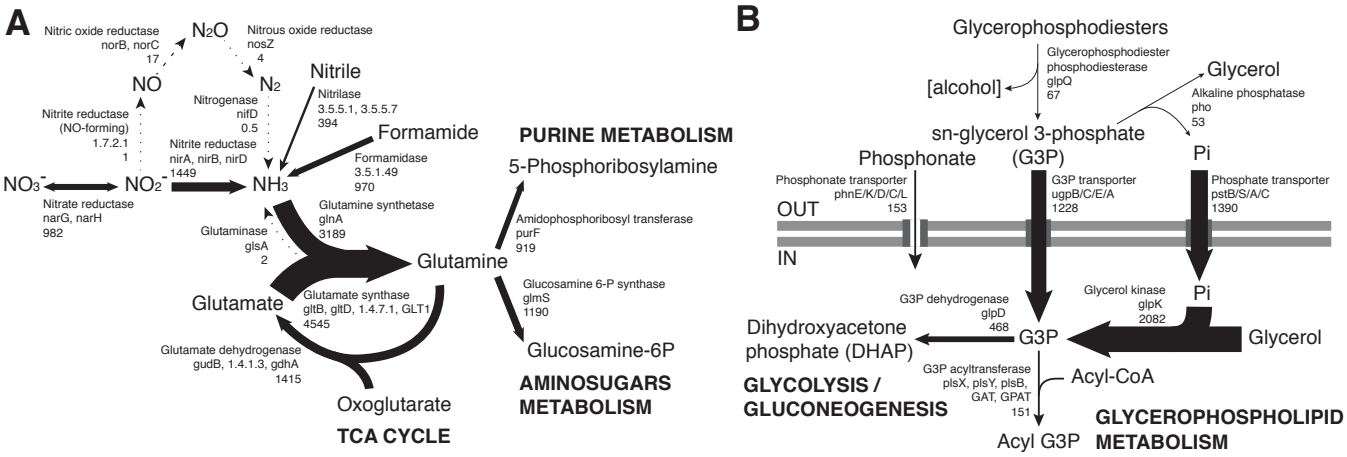

Figure 4