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**Evidence of species recruitment and development of hot desert hypolithic
communities**

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Abstract

Hypoliths, photosynthetic microbial assemblages found underneath translucent rocks, are widely distributed within the western region of the Namib Desert and other similar environments. Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to assess the bacterial community structure of hypoliths and surrounding soil (below and adjacent to the hypolithic rock) at a fine scale (10 m radius). Multivariate analysis of T-RFs showed that hypolithic and soil communities were structurally distinct. T-RFLP derived OTUs were linked to 16S rRNA gene clone libraries. Applying the ecological concept of ‘indicator species’, 6 and 9 indicator lineages were identified for hypoliths and soil, respectively. Hypolithic communities were dominated by cyanobacteria affiliated to Pleurocapsales, whereas actinobacteria were prevalent in the soil. These results are consistent with the concept of species sorting and suggest that the bottom of the quartz rocks provide conditions suitable for the development of discrete and demonstrably different microbial assemblages. However, we found strong evidence for neutral assembly processes, as almost 90% of the taxa present in the hypoliths were also detected in the soil. These results suggest that hypoliths do not develop independently from microbial communities found in the surrounding soil, but selectively recruit from local populations.

Introduction

The Namib Desert in South West Africa is considered to be the world's most ancient desert and has substantially varied ecotopes including gravel plains, dunes, inselbergs, escarpments, and playas (Eckardt and Drake, 2010). This desert spans a longitudinal distance of over 200 km, stretching from the western coastline to the eastern mountains along the Tropic of Capricorn. The Namib has been classified as an arid zone with some regions demonstrating hyperarid characteristics (Eckardt *et al.*, 2012). The desert surface is subject to wide temperature fluctuations (from 0 °C to as high as 50 °C) with a general increase from the coast inland. Rainfall patterns within this desert are scant and erratic, with long periods of aridity (Eckardt *et al.*, 2012).

The undersides of rocks in climatically extreme deserts, such as the Namib, act as a refuge for microorganisms (defined as “hypoliths”) and their community (the “hypolithon”) (Chan *et al.*, 2012; Pointing and Belnap, 2012). The overlying rock creates a favorable sub-lithic microhabitat where microorganisms benefit from greater physical stability, desiccation buffering, increased water availability and protection from UV fluxes (Pointing *et al.*, 2009; Cowan *et al.*, 2010). As they are typically dominated by primary producers (Cockell and Stokes, 2004; Wood *et al.*, 2008) hypolithic communities are thought to be significant contributors to regional carbon and nitrogen inputs (Burkins *et al.*, 2001; Cowan *et al.*, 2011).

Previous studies have suggested that hypolithons develop independently from surrounding soil communities (Warren-Rhodes *et al.*, 2006; Pointing *et al.*, 2007; Davila *et al.*, 2008; Tracy *et al.*, 2010). However data on the mechanisms of community assembly leading to site-to-site

variations (beta diversity) in community composition in deserts remain scant. Recently, Caruso *et al.* (2011) reported that deterministic and stochastic processes interact in the assembly of hypolithons on a global scale. However, the drivers of bacterial beta-diversity are known to depend both on spatial (Martiny *et al.*, 2011) and temporal scales (Langenheder *et al.*, 2012; Lindström and Langenheder, 2012). For example, dispersal limitation was found to drive Nitrosomonadales beta-diversity at the scale of an individual marsh (Martiny *et al.*, 2011). In direct contrast, the environment was the most important factor in explaining differences between these communities across regional and continental scales (Martiny *et al.*, 2011). These differences highlight the need to identify the patterns and mechanisms that shape bacterial community composition in different habitat types and at different spatial scales.

Here, we apply the ecological concept of “indicator species” (Dufrene and Legendre, 1997) to interrogate the process behind hypolithic community assembly at a microscale (10 m radius), and present strong evidence that in the Namib Desert recruitment from soil sources supports hypolithic community assembly. We predict that if deterministic processes are strong, hypoliths and surrounding soil should demonstrate greatly dissimilar bacterial communities (specialists). If the effect of the environment is limited, both hypolith and surrounding soil should contain similar bacterial communities (generalists).

Results and discussion

The comparative bacterial composition of hypolithic and nearby soil samples at a desert site in the hyperarid Namib Desert was assessed using T-RFLP analysis and clone libraries (see Supporting Information for materials and methods). A total of 98 T-RFs were obtained,

ranging from 23 to 44 OTUs for the individual samples. When averaged for the different sample types, hypoliths and surrounding soil contained similar numbers of OTUs, with values of 22.0 [± 4.7 (SD)], 25.5 [± 7.2 (SD)] and 30.3 [± 6.7 (SD)] for hypoliths, open soil and sub-lithic soil, respectively. Shifts in OTU composition (β -diversity) revealed that 5 OTUs were unique to the hypoliths, 10 were unique to the open soil and 29 were unique to sub-lithic soil (Fig. S1). In total, 38 OTUs (38 % overlap) were shared between hypolith and soil samples.

When bacterial community patterns were visualized by NMDS of Bray Curtis similarities, communities grouped separately according to their habitat (Fig. 1). Similar results were obtained after accounting for the unequal number of samples by applying a random resampling procedure (Fig. S2). When habitat type, depth and the interaction between both factors were assessed in an *adonis* model (PERMANOVA analysis), habitat was found to have a significant effect ($F_{2,28}=4.82$, $P=0.001$). Each group was clearly distinct (hypoliths vs. sub-lithic soil $R^2=0.26$, $P=0.001$; hypoliths vs. open soil $R^2=0.30$, $P=0.001$; sub-lithic vs. open soil, $R^2=0.08$, $P=0.02$); that is, the overlying quartz rocks not only influenced the hypolithon but also the soil bacterial community below the rock. Although differences between hypolithic and soil bacterial community structure have been reported in polar deserts (Pointing *et al.*, 2009; Khan *et al.*, 2011), similar observations have not been reported for hot desert communities. In contrast to previous studies of microbial communities (Zhou *et al.*, 2002; Ge *et al.*, 2008) no spatial variation on vertical axes was observed, although these studies were performed on a broader scale and bacterial community patterns are known to depend on both spatial and resource factors (Zhou *et al.*, 2002; Martiny *et al.*, 2011).

In order to relate OTU abundance and habitat type, a multivariate regression tree (MRT) analysis was performed. Habitat type alone explained 10 % of the variation observed. Indicator OTUs identified using the IndVal indexes were mainly responsible for the topology of the tree (Fig.2a) suggesting that these specialist lineages represented ecological indicators of the prevailing environmental. Overall, 6 and 9 OTUs were found to be statistically significant indicators of the hypoliths and surrounding soil, respectively ($P < 0.05$) (Fig.2b).

Clone libraries yielded a total of 85 unique, non-chimeric sequences, of which 33 and 52 clones were sequenced from hypolith and soil, respectively (Table S1). Phylogenetic analysis of the clone libraries was consistent with multivariate analysis of the T-RFLP profiles. Both F_{ST} and P tests were significant (not shown), indicating a lower genetic diversity within each community than for two communities combined and that the different communities harbored distinct phylogenetic lineages (Martin, 2002). Rarefaction curves and Chao 1 estimates indicated that sampling had approached an asymptote only for hypoliths (Fig.S3). In spite of the relatively low number of clones sampled this is not unexpected since previous studies have shown low phylogenetic diversity in hot desert ecosystems (Wong *et al.*, 2010). The majority of the clones displayed homology to sequences retrieved from hot hyperarid deserts (Table S1). Nonetheless, only 6 OTUs showed identity values higher than 97 %, indicating that the majority of sequences might represent novel taxa.

Soil samples were dominated by the phyla Actinobacteria (49%) and Proteobacteria (21%). Acidobacteria, Cyanobacteria, Bacteroidetes and Chloroflexi phylotypes were detected in lower numbers (Fig. S4, Fig. S5). Members of these phyla are generally among the most common

inhabitants of soils (Fierer and Jackson, 2006; Jones *et al.*, 2009; Lauber *et al.*, 2009). Clones derived from hypoliths were affiliated to the phylum cyanobacteria (85%) dominated by *Chroococidiopsis* lineages (order Pleurocapsales), although members of the orders Oscillatoriales, Stigonematales, and Chroococcales were also observed. *Chroococidiopsis* has been identified as one of the common primary producers occurring in both hot and cold deserts (Tracy *et al.*, 2010; Bahl *et al.*, 2011; Caruso *et al.*, 2011; Lacap *et al.*, 2011). Other phyla represented in the hypolithic clone library included Acidobacteria (2.9%), Proteobacteria (2.9%), Actinobacteria (2.9 %) and unclassified bacteria (3%). A total of 60 (out of 98) T-RFLP-defined OTUs were matched to 16S rRNA gene sequences resulting in an overall assignment of 61 %.

We found that hypolithic and surrounding soil indicator species were identified as cyanobacteria and actinobacteria, respectively. If indicator lineages play a pivotal ecological role within the habitat (Auguet *et al.*, 2010), these results support the view that cyanobacteria are among the most important functional groups in hypoliths (Cowan *et al.*, 2011). Cyanobacteria are ubiquitous in most terrestrial habitats, and have central ecological roles in energy transduction, nitrogen fixation and as pioneer species (Whitton and Potts, 2000).

Only 5 OTUs were exclusive to hypolithic samples and the most abundant OTUs were present in both soil and hypolithic samples. This is somehow consistent with neutral theory predictions (Hubbell, 2001) that assume species are ecologically equivalent. Thus, the compositions of local communities are regulated only by chance without considering deterministic factors (intra-specific competition or niche differentiation). Although these assumptions are still controversial, there is empirical evidence that both deterministic and stochastic processes shape the structure of

microbial communities (Dumbrell *et al.*, 2010; Ofiteru *et al.*, 2010; Caruso *et al.*, 2011; Langenheder and Szekely, 2011). Notably, a global-scale study of hypolithic communities found that neutral models failed to show evidence of deterministic processes when cyanobacteria and heterotrophic bacteria were analyzed separately, whereas species co-occurrence was non-random when both groups were analyzed together (Caruso *et al.*, 2011). The global study of Caruso and co-workers identified demographic stochasticity as a major factor influencing community assembly, and here we present evidence that stochasticity also plays a pivotal role in local community assembly. Since 88 % of the OTUs observed in hypolithic community samples were also found in soil it is most likely that a great proportion of taxa that “seeded” hypoliths were recruited from the surrounding soil. It is also possible that a common source (e.g., bio-aerosols) seeded both soil and hypolithic communities. In any case, under the assumptions of neutral theory it might be expected that taxa composition and abundance should be approximately the same in hypoliths and in soil (Sloan *et al.*, 2006; Ostman *et al.*, 2010). As has been observed previously in rock pools seeded by rainfall water (Langenheder and Szekely, 2011) or lakes seeded by soils (Crump *et al.*, 2012), we found that most abundant taxa in the soil were also present in hypoliths albeit in lower abundance (Fig. 3). Nevertheless, this was not always the case as demonstrated by the presence of indicator species (Fig. 2b). Consequently, the neutral theory failed to explain all the variation found in the bacterial community structure. In fact, cyanobacteria and actinobacteria were over-represented in hypoliths and surrounding soil, respectively, suggesting that deterministic processes (habitat filtering) are also important.

We suggest three non-exclusive reasons for the relatively weak deterministic effect. Firstly, it could reflect a limitation of the technique (i.e., T-RFLP), as it is well known that fingerprinting

methods only target the most abundant taxa (Bent and Forney, 2008). Secondly, critical deterministic elements of local environmental in hypoliths and surrounding soil at the Namib study site may not differ significantly (temperature and %RH values are shown in Fig. S6). Finally, high dispersal rates (source-sink dynamics) (Cottenie, 2005) could buffer the effect of selection by continued homogenization of the communities involved. Indeed, there was a high degree of overlap between the soil and hypolithic communities (Fig. 3, Fig. S1). It is important to note, however, that non-neutral processes such as intra-species interactions, invariance under assemblage or the complexity of ecological interactions and the ‘melting’ of competitive hierarchies can generate neutral patterns (Alonso *et al.*, 2006). Clearly, more focused research is required in order to explain the differences in microbial community structure between hypoliths and soil.

Metacommunity studies typically relate assembly processes to the entire community and do not take into account different categories of species. However, it has been shown for aquatic bacteria that habitat specialists and generalists have different population dynamics (Shade *et al.*, 2010). Co-occurrence patterns were also found for soil microbial communities (Barberan *et al.*, 2011). More important, habitat generalist and specialist have been shown to differ in their respective contributions to ecosystem functioning (Gravel *et al.*, 2011).

In conclusion, the presence of generalist lineages indicates that Namib hypolithic bacterial communities did not develop independently from the surrounding soil. This is in contrast to some hyperarid Antarctic hypoliths where cyanobacteria-dominated hypolithon occurs in soils where cyanobacterial signatures were undetectable by sequence analysis of environmental clone

libraries (Pointing *et al.*, 2009). Similarly in the hyperarid Atacama Desert hypoliths occur in soils devoid of recoverable cyanobacteria, although other reservoirs of cyanobacteria exist in this desert within deliquescent minerals (Davila *et al.* 2008; de los Rios *et al.* 2010; Weirzchos *et al.* 2012). The significant fog-moisture input to our Namib study site may be a factor affecting microbial diversity in soil reservoirs, and the extent to which aridity affects this will be a fruitful area for future work. In our study we provide empirical evidence that cyanobacteria are indicator species (specialists) for hypoliths, suggesting that both habitat filtering and stochastic processes shaped the assembly of hypolithic bacterial communities in the Namib. Since specialist assemblages seem to be more productive (Gravel *et al.*, 2011) and more susceptible to extinction than generalists when habitat conditions are altered (Tilman *et al.*, 1994), these results have implications for habitat conservation in drylands that support hypoliths. Our study suggests that future investigations of hypoliths could exploit our finding that cyanobacteria are indicator taxa and focus more closely on this component to infer ecological patterns.

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Figure Legends

Fig. 1

nMDS ordination plot (Bray-Curtis distance matrix) of T-RFLP profiles for soil- and hypolith-derived samples. The quality of the ordination is indicated by a low-stress value.

Fig.2

Multivariate regression tree (MRT) analysis(a). The model explained 10% of the variance in the whole data set. Bar plot under each leaf [magnified in (b)] show the contribution of the different indicator species. The number of samples included in the analysis is shown under bar plots.

Fig.3

Bubble plot. Each bubble represents one T-RF (columns) and is sized according to its relative abundance in the sample (rows).

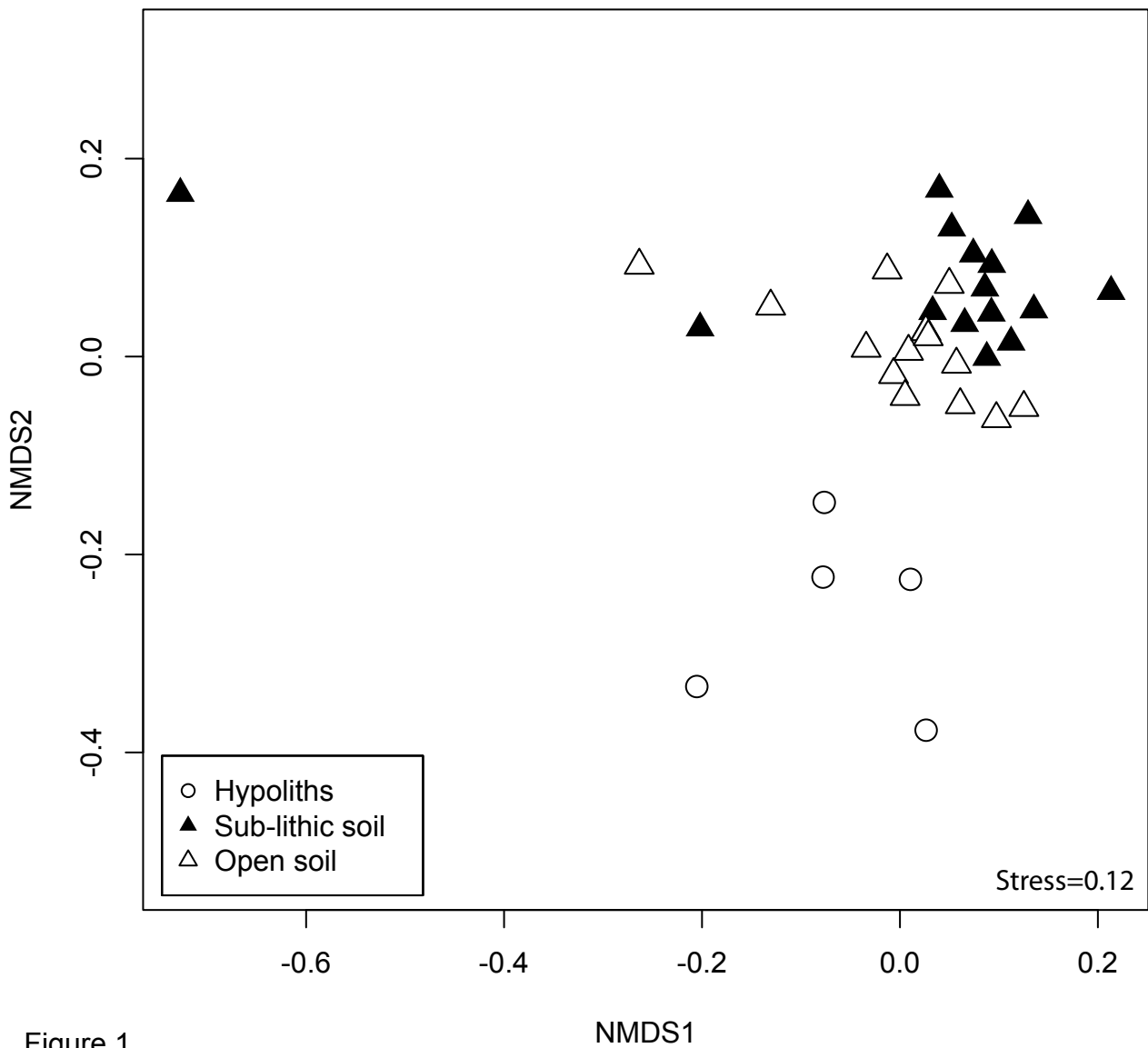


Figure 1

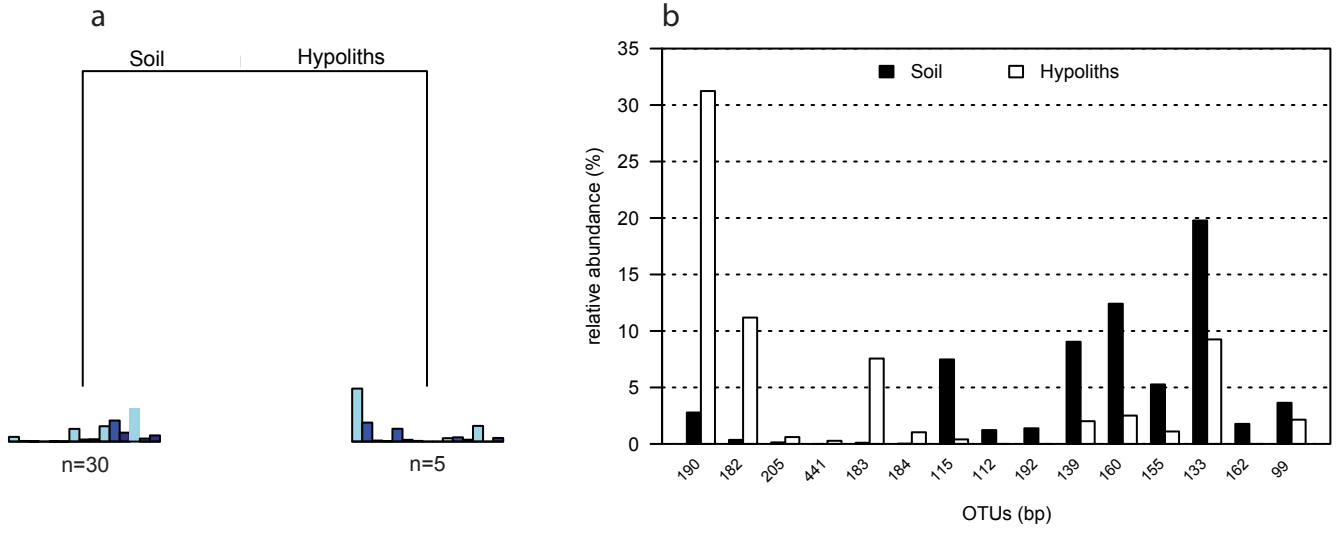


Figure 2

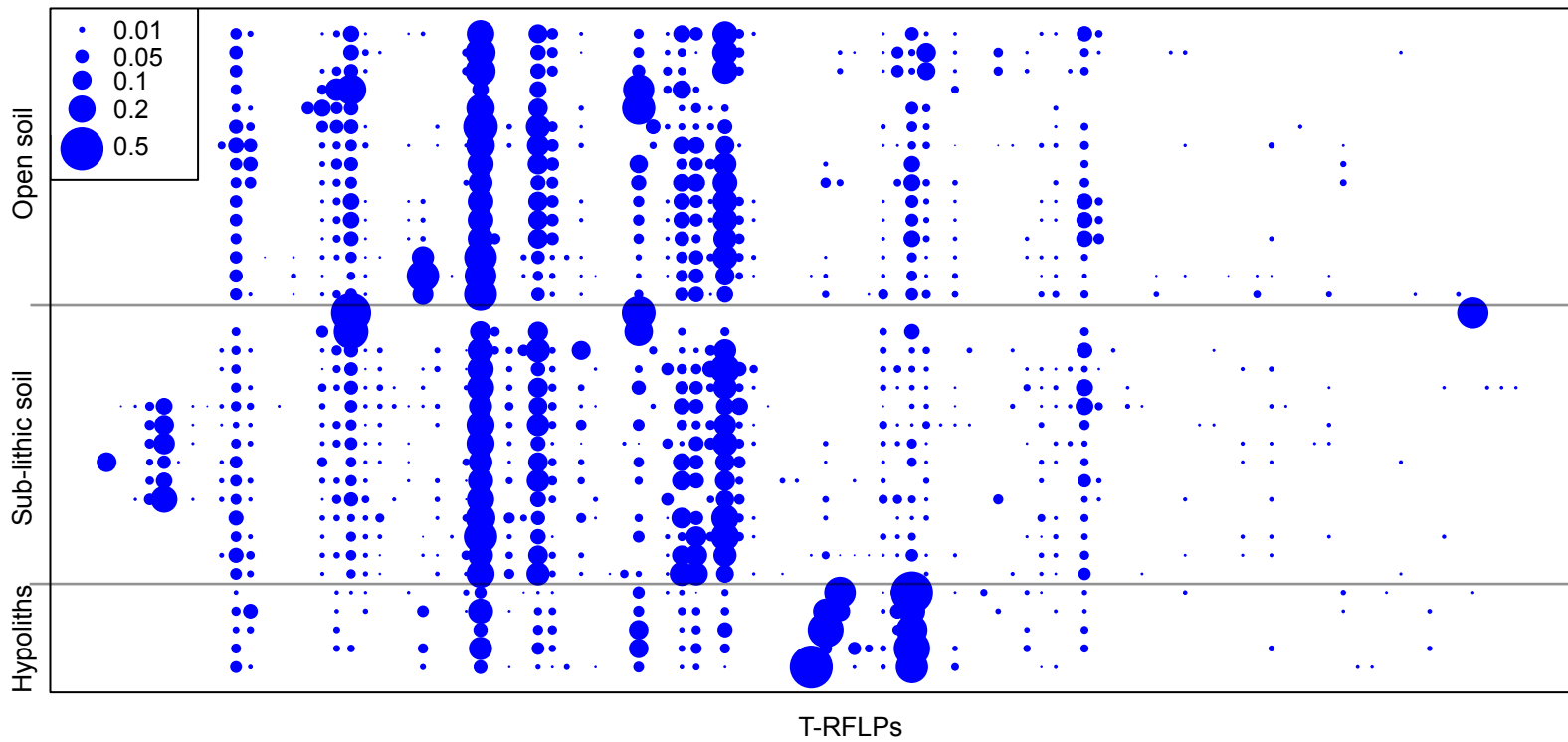


Figure 3