Human disturbance drives differential diversity patterns of microbial communities in hypogean habitats

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Abstract

The metacommunity framework has been rarely adopted to investigate the underlying ecological mechanisms shaping microbial communities. With the aid of advanced molecular techniques, we investigated sediment communities of Fungi, Bacteria and Archaea in four Italian show caves aiming to disentangle the effects induced by tourists on species richness and composition from environmental filtering and dispersal driven mechanisms. We modelled community changes against human disturbance —measured as the distance from the tourist path—demonstrating that the presence of visitors in caves decreases fungal species richness and causes species replacement in Bacteria and Archaea. Environmental filtering affects species richness and composition of Fungi and species richness of Archaea, while a minor role was played by dispersal, influencing only species richness in Fungi. We provide new perspectives on the dynamics of microbial communities under human disturbance suggesting that a proper understanding of the underlying selective mechanisms requires a comprehensive and multi-taxonomic approach.

1. INTRODUCTION

Anthropogenic disturbance on natural ecosystems is growing in frequency and magnitude worldwide, with consequent negative repercussions on biodiversity (Ceballos et al., 2015; Pereira et al., 2012; Pimm et al., 2014; Wagner et al., 2021). However, disentangling the underlying mechanisms that determine changes in biotic communities at the local scale is often hampered by habitat-dependent confounding effects (Cardinale et al., 2018; Gonzalez et al., 2016; McGill et al., 2015; Vellend et al., 2017). Notably, these limitations are evident in microbial communities due to rapid evolution (Niehus et al., 2015), high taxonomic richness (Shoemaker et al., 2017), functional redundancy (Curtis & Sloan, 2004), and dormancy (Locey et al., 2020). Insights into the main processesdetermine changes in local communities following external perturbations may be obtained by examining variation in species richness and composition in a metacommunity ecology perspective (Jurburg et al., 2021; Kinnunen et al., 2016). Metacommunity theory emphasises how species richness of a community can be explained by two coexisting limiting forces, summaried into two main paradigms, the island and the trait-environment paradigm. The former states that number of species in a community depends on dispersal limitation, imposed by both geographical barriers and species dispersal capacity (MacArthur & Wilson, 1967); the latter identifies niche assembly rules as the major force in selecting species, emphasising environmental filters based on species morphological, physiological and behavioural traits (Cody et al., 1975). Despite its potential capacity of disentangling the main selective mechanisms

of biotic communities under human disturbance (Leibold & Chase, 2017), this framework has been rarely applied microbial communities (Jurburg et al., 2021).

Being characterised by highly predictable gradients in their environmental conditions, simplified trophic webs, climatic stability, and spatial confinement (Culver & Pipan, 2019; Poulson & White, 1969), subterranean ecosystems represent ideal ecological laboratories in this regard (Mammola, 2019). Among the main threats affecting subterranean ecosystems (Mammola et al., 2022), the ever-increasing conversion of natural caves into tourist attractions, i.e. the so-called 'show caves', imposes significant ecological pressure on the subterranean ecosystem (Cigna, 2016). Although this phenomenon provides both an economic and social opportunity to local development (Allan et al., 2015; Kim et al., 2008; Shavanddasht et al., 2017), the presence of visitors throughout the year significantly impacts the subterranean environment. Well-documented effects on abiotic component of the subterranean ecosystems include changes in microclimate (e.g. Šebela et al., 2015, 2019; Addesso et al., 2022a), carbon dioxide concentration (e.g. Lang et al., 2015, 2017; Addesso et al., 2022a), and rock composition (e.g. Addesso et al., 2019), with cascade effects on the subterranean fauna (e.g. Nicolosi et al., 2021; Pacheco et al., 2021) and energy fluxes (e.g. Addesso et al., 2022b; Fernandez-Cortes et al., 2011), also induced by the proliferation of alien photosynthetic microorganisms caused by the installation of artificial light at different intensities (e.g. Havlena et al., 2021; Piano et al., 2015) and duration (e.g. Borderie et al., 2014; Piano et al., 2021). In addition, evidence in literature indicates tourists as vehicles of microbial species alien to the cave, which represent a source of biological pollution for the cave air (Martin-Sanchez et al., 2014; Porca et al., 2011), water (Ando & Murakami, 2020; Moldovan et al., 2020), soil (Kukla et al., 2018; Mammola et al., 2017) and speleothems (Bercea et al., 2019). Also, their outbreaka can cause significant biochemical and biophysical degradation (Saiz-Jimenez et al., 2012) and perturbations of resident communities by potentially competing with, and even overriding, autochthonous species (Alonso et al., 2019; Griffin et al., 2014). However, the consequences of the touristic pressure on microbial communities in subterranean ecosystems have never been tested within a metacommunity framework .

With the aid of advanced molecular techniques, we examined changes in species richness and composition of three microbial components that naturally inhabit cave sediments, i.e. Fungi, Bacteria and Archaea, in four Italian show caves, facing anthropogenic disturbance. By adopting a replicated factorial design, we tested: i) to what extent species diversity and composition of microbial communities is determined by dispersal-driven mechanisms and/or environmental filtering; and ii) whether tourists' disturbance influences local microbial communities. We hypothesised that: i) human-driven mechanisms represent the main selective force in microbial communities; and ii) propagules vehiculated by tourists would cause a replacement of the original species, thus emphasising the turnover process.

2. MATERIALS AND METHODS

2.1. Sampling design

We adopted a replicated two-factorial sampling design wherein sediment samples were repeatedly sampled at progressive distance from the tourist path and at progressive distance from the entrance in four Italian show caves (Fig. 1).

In each cave, we identified four sampling areas at progressive distance from the cave entrance: Section I to IV, with Section I being the closest to the entrance and Section IV the furthest. Sections I, II and III were located in areas open to the public (touristic areas, T), while Section IV was located in an area closed to the public in the deepest part of the cave (control area, C). The length of each Section was one third of the tourist path length. Within each sampling section of the touristic part, we identified three sampling transects placed at progressive distance from the tourist path: the High Pressure (HP) transect at 0-3 m from the tourist path; the Medium Pressure (MP) transect at 3 to 5 m from the tourist path; and the Low Pressure (LP) transect at > 5 m from the tourist path. In the control area (Section IV), three sampling transects were randomly placed to obtain a representative view of the natural conditions of the cave.

Along each transect, we collected 9 random replicates of alluvial sediment within 9 squares of 10 cm^2. Sediment was collected on the ground up to 3 cm depth with sterile Falcon® tubes (50 mL). Samples were

preserved in a thermal bag until the arrival at the laboratory, where the 9 replicates for each sub-transect were pooled together and homogenised. In this way, we obtained a total of 12 samples (4 transects × 3 sub-transects) for each show cave. We focused on soil as it proved to be the compartment with the highest microbial diversity (see Alonso et al., 2019). As seasonal differences have been observed in the subterranean microbiota (Mammola et al., 2017), samplings were performed in Summer, between June and September 2020, in order to avoid possible seasonal variations that could mask the effect of tourism on the soil microbiota.

Sediment samples were sieved, under sterile conditions, by removing coarse rock debris. Their physical and chemical properties were evaluated by measuring pH, concentration of Organic Carbon, total Nitrogen and the percentage of sand (%Sand), silt (%Silt) and clay (%Clay) with standard protocols by Regione Piemonte - Laboratorio Agrochimico - Settore Fitosanitario e Servizi Tecnico-Scientifici.

2.2. Metagenomic DNA extraction, amplicon sequencing and bioinformatics

Metagenomic DNA was extracted from 0.5 g of sample using Qiagen DNeasy PowerSoil Pro Kit (Carlsbad, CA, USA). The Internal Transcribed Sequence 1 ribosomal region (ITS1) and hypervariable region V4 of 16S ribosomal gene were targeted to assess the fungal and prokaryotic community composition, respectively. The ITS1 region was amplified using barcoded primers ITS1F/ITS2, suitable for shorter read length (Smith & Peay, 2014), while for the V4 region of 16S, barcoded F515/R806 primer set was used according to Caporaso et al. (2012). PCR reactions consisted of 1 µL of each primer, 12.5 µL of Taq DNA Polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA), 9.5 µL of nuclease-free water (Sigma–Aldrich, St. Louis, MO, USA) and 5 ng of DNA for a total volume of 25 µL and occurred in an automated thermal cycler (BioRad, Hercules, CA, USA),. The ITS1 locus and V4 region were amplified according to Coleine et al. (2021). Amplicons were quantified by a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA) and then pooled. Paired-end sequencing (2 × 300 bp) was carried out on an Illumina MiSeq platform at the Edmund Mach Foundation (San Michele all'Adige TN, Italy). Demultiplexed ITS and 16S sequence datasets were processed using AMPtk (Palmer et al., 2018) v.1.5.1 software. Briefly, barcodes/indexes and primer sequences were removed from raw data. Reads were subjected to quality trimming to a maximum of 250 bp, discarding those shorter than 100 bp; sequencing artefacts were dropped by using USEARCH v.9.1.13 with default parameters (Edgar, 2010). Sequence quality filtering was performed with the expected error parameter of 0.9 (Edgar & Flyvbjerg, 2015); the cleaned reads were merged and clustered at 99% similarity using VSEARCH (Rognes et al., 2016) v.2.15.1, with DADA2 (Callahan et al., 2016). Global singletons and rare taxa (<5 reads) were skipped as likely false positives due to sequencing errors (Lindahl et al., 2013). Finally, taxonomic identification was performed with hybrid database SINTAX/UTAX (Edgar, 2010).

2.3. Species richness and composition

In the subsequent analyses, we considered the number of Amplicon Sequence Variants (ASVs) obtained from the ITS as a proxy of the fungal species richness, while the number of ASVs obtained from the 16S were split into Archaea and Bacteria based on the procedure that assigns the Domain. Being interested in deviations from natural conditions of caves, for each cave we calculated the ratio between the ASV richness of each sample collected in the tourist part and the ASV richness of the sample collected in the control area, which is located in the deepest part of the cave, closed to the public (hereafter ASV-ratio).

We then obtained the variation in ASV composition in Fungi, Bacteria and Archaea assemblages by calculating their pairwise dissimilarity among samples by means of the complement of the Sørensen index, which ranges from 0 (samples are composed exactly by the same taxonomic entities) to 1 (samples do not share any taxonomic entity). We used the function 'beta' in the *BAT* package (Cardoso et al., 2015), which allowed us to decompose the total dissimilarity (total β-diversity) into the contribution of its additive components (β-replacement and β-richness), following the partitioning framework independently developed by Podani & Schmera (2011) and Carvalho et al. (2012). The two measures reflect different mechanisms shaping species composition, with β-replacement ($\beta_{\rho\epsilon\pi\lambda}$) reflecting dissimilarity explained by species replacement and β-richness ($\beta_{\rho\iota\varsigma\eta}$) reflecting dissimilarity explained by species loss/gain (richness differences).

For each cave we calculated the dissimilarity measures between each sample collected in the tourist part with

respect to the community sampled in the control area. Thanks to this approach, we obtained a measure of the distance of each sample from the control communities.

2.4. Data analysis

We performed all statistical analyses in R (R Core Team, 2021).

In a first step, we analysed the chemical and physical parameters of collected sediments by means of a Principal Component Analysis (PCA) with the function 'prcomp'. To detect possible shifts in physical and chemical parameters of collected sediments among transects (HP – MP – LP – C), sections (I – II – III – IV) and caves (Bossea, Caudano, Pertosa-Auletta and Vento), we applied a Permutational Multivariate Analysis of Variance (PERMANOVA, Anderson, 2001) based on Euclidean distances, specifying transects, sections and caves as factors, with the function "adonis" from the *vegan* package (Oksanen et al., 2019). Statistical significance was tested via 9,999 random permutations.

Before proceeding with statistical models, we converted the two factors of our two-factorial sampling design, namely sections and transects, into ordinal variables in order to effectively represent a gradient of progressive distance from the cave entrance (Sections: I = 1, II = 2, III =3) and from the tourist path (Transects: HP = 1, MP = 2, LP = 3). Although air circulation in caves can be extremely complex (Badino, 2018), we here considered the distance from the cave entrance (Dist_entrance) as a proxy of the dispersal of propagules from the external environment vehiculated by air, i.e. dispersal selective mechanisms. The distance from the tourist path (Dist_path) was intended as a proxy of human disturbance determined by visitors, i.e. disturbance selective mechanisms. Physical and chemical parameters of collected sediments, namely granulometric composition (% silt, sand and clay) and concentrations of organic carbon and nitrogen, were considered as proxy of the environmental selection, i.e. habitat filtering. More in detail, we obtained the dissimilarity based on a Bray-Curtis distance of sediment composition of each sample collected in the tourist part of each cave from the sediment composition of the sample collected in the control area. Therefore, the obtained variable (Substrate) summarises the differences in the substrate conditions in touristic sections compared to the control area.

The effect of these three variables and their interactions was tested on the: (i) ASV-ratios of Bacteria, Archaea and Fungi; ii) dissimilarity measures (i.e. β-replacement and β-richness) of Bacteria, Archaea and Fungi, by means of Generalized Linear Mixed Models (GLMMs). In a preliminary step, we graphically checked for potential outliers in our dependent variables (Zuur et al., 2010). Statistical models were performed with the function 'glmmTMB' from the *glmmTMB* package (version 1.1.2.3, Brooks et al., 2017). We assumed a Gamma error distribution for the set of models performed on the ASV-ratios and a Beta error distribution for the set of models performed on the dissimilarity measures. To account for the spatial dependency of samples within the same show cave, a cave identifier (CaveID) was incorporated as a random factor. The interaction factors were retained only if they significantly contributed to the fit of the model based on the outcomes of the 'anova' function. Model validation was performed with the function 'check_model' from the *performance* package (Lüdecke et al., 2021) as well as by visually inspecting the distribution of the residuals (Zuur et al., 2016).

3. RESULTS

3.1. Metabarcoding data

The ITS1 dataset generated 5,793,980 raw sequence reads, resulting in 5,458,895 gene quality-filtered reads, ranging from 1,252 up to 540,803 per sample. After singletons and rare taxa (<5 reads) removal (1,108 out of 10,595 ASVs total), a total of 9,487 high-quality ASVs were obtained. A total of 5,453,881 raw reads were generated from 16S rDNA dataset and accounted for a total of 4,806,902, which were grouped into 47,367 ASVs (out of a total of 65,037 ASVs) after quality filtering, with sequencing depths between samples ranging from 2066 to 265,442 reads.

The microbial community was dominated by fungi (51% of the entire datasets), where the phylum Ascomycota dominated (72%), followed by Basidiomycota (15%). Bacteria dominated the prokaryotic diversity, among

which the most abundant phyla were Proteobacteria (40%), Acidobacteria (16%), and Actinobacteria (14%). The domain Archaea was much less represented, encompassing only the 2% of ASVs across the dataset, being constituted mainly by the phylum Thaumarchaeota (62.5%), while Euryarchaeota and Crenarchaeota were present at lower percentage (22.2 and 12.4%, respectively).

3.2. Sediment analysis

The results of the PCA showed that the first three axes explain 90% of the total variance (Axis 1 = 52.1%; Axis 2 = 26.4%; Axis 3 = 11.5%; Axis 4 = 0.08%; Axis 5 = 0.02%). The variable loadings to each axis demonstrate that total Nitrogen concentration, %Clay, pH and %Sand equally contribute to the variance of the first axis (Tab. 1). While the contribution of total Nitrogen concentration and %Clay is positive, the contribution of pH and %Sand is negative. Regarding the second axis, most of the variance is explained by the %Sand and %Silt, with positive and negative contribution, respectively (Tab. 1). The variance of the third axis is mostly explained by the concentration of organic carbon, which shows a negative contribution (Tab. 1). Results of the PERMANOVA performed on the physical and chemical parameters of collected sediments (Fig. 2) revealed significant differences among caves ($F_{3,31}$ = 55.4, P = 0.001), but not among transects ($F_{3,31}$ = 1.81, P = 0.165) and sectors ($F_{2,31}$ = 0.856, P = 0.485).

3.3. ASV-ratios

The ASV-ratios were generally lower than one (Tab. S1), pointing out that ASV richness values observed in the tourist areas were lower than in the control area.

The results of the models (Tab. 2 and Fig. 3a) performed on the ASV-ratios showed that the ASV-ratio of Fungi was negatively affected by the distance from the tourist path and positively affected by its interaction with the distance from the cave entrance (Fig. 3a). No response was recorded for Bacteria and Archaea in relation to these two parameters (Fig. 3a). In addition, both Fungi and Archaea were negatively affected by the increasing dissimilarity of sediment composition (Fig. 4a). In other words, the number of ASV in the touristic part of Archaea and Fungi declined when sediment composition differed from the control area.

3.4. Beta diversity

The total β-diversity was extremely high (Fungi = 0.96; Bacteria = 0.92; Archaea = 0.93), with the β-richness component being dominant (Fungi = 0.55; Bacteria = 0.54; Archaea = 0.65) over the β-replacement component (Fungi = 0.41; Bacteria = 0.38; Archaea = 0.28). The results of the models (Tab. 2b-c and Fig. 3b-c) performed on the two components showed an effect of the distance from the tourist path on the β-replacement of the prokaryotic component, i.e. Bacteria (Fig. 3b) and Archaea (Fig. 3b). Both groups showed a significant decline of the β-replacement at increasing distance from the cave entrance (Fig. 3b). In parallel, we observed an increase in the β-richness component for Archaea (Fig. 3c) and, to a lesser extent, for Bacteria (Fig. 3c). Our results also highlighted a nearly significant effect of the cave depth on the β-replacement (Fig. 3b), but not on β-richness (Fig. 3c), of Fungi with decreasing species turnover at increasing distance from the cave entrance (Tab. 2b). In addition, β-diversity of Fungi was significantly affected by the sediment composition (Tab. 4), with increasing β-replacement (Fig. 4b) and declining β-richness (Fig. 4c) at increasing dissimilarity in sediment composition from the control areas.

4. DISCUSSION

The conversion of caves into touristic attractions has major impacts on subterranean ecosystems (Fernandez-Cortes et al., 2011; Mulec, 2014; Pulido-Bosh et al., 1997), and causes large-scale indirect anthropogenic disturbances, such as land use change (Jiménez-Sánchez et al., 2008) and climate change (Mammola et al., 2019). Yet, studies show inconsistent responses of the microbial communities to human disturbance, pointing out either a positive (Mammola et al., 2017; Marques et al., 2016), negative (Alonso et al., 2019; Shapiro & Pringle, 2010) or variable effect (Bercea et al., 2019; Mulec & Oarga-Mulec, 2012). These controversial outcomes are likely attributed to differences in the examined groups and to the environmental variations across different caves (Pfendler et al., 2018, 2019). Examining human-induced changes in subterranean microbial communities across different caves is often hampered by the great differences in local species composition,

which is strictly influenced by geographic and geological features such as cave size, morphology, and water dynamics (Saiz-Jimenez, 2012). To overcome these limitations, we simultaneously analysed the response of the three main microbial groups inhabiting caves, namely Fungi, Bacteria and Archaea, combining the data collected across multiple show caves. By relating both species richness and community composition in the tourist parts to those observed in control areas, we could depict the human-induced effects on microbial communities, without confounding factors due to local environmental features. By adopting a metacommunity framework, we disentangled the effect of visitors on subterranean microbial communities from the habitat filter and the dispersal limitation for the first time.

Our results pointed out an effect of visitors on the diversity and composition of microbial communities in show cave sediments, but with different outcomes depending on the examined microbial group. When separately analysing the response of the three groups, our results pointed out that human disturbance does not affect the species richness of the prokaryotic component, as demonstrated by the absence of a significant response of the models fitted on the ASV-ratios. However, we clearly showed that visitors influence the composition of microbial communities of Bacteria and Archaea by causing a turnover of species as demonstrated by the decline of β-replacement at increasing distance from the tourist path. These results are in accordance with the outcomes of a meta-analysis testing the response of microbial communities to disturbance, which showed only a weak response in terms of species richness but the effects were evident on the community composition (Jurburg et al., 2021). The observed species turnover on the tourist path could be ascribed to the replacement of resident species by propagules of microorganisms vehiculated by visitors' shoes and clothes, which are spread around while visiting the cave (Mulec, 2014; Saiz-Jimenez, 2012; Zhelyazkova et al., 2020), similarly to what observed by other authors (Alonso et al. 2019; Dong et al., 2020; Ikner et al. 2007).

The response of Fungi showed a U-shaped response to the human disturbance. The ASV-ratio in samples close to the path was similar to those found in control sites, but decreased significantly at intermediate distance. This outcome suggests that visitors increase fungal species richness either directly —by vehiculating propagules of novel species (Jurado et al., 2021; Mammola et al., 2017; Taylor et al., 2013)— or indirectly —by introducing high amounts of organic matter, beyond the lampenflora biomass proliferating in the visited paths due to the lighting system, that favour more competitive species (Jurado et al., 2010; Marquez et al., 2016; Addesso et al., 2020). However, in sampling sites located deeper in the cave, the pattern is reversed, with increasing ASV-ratio at increasing distance from the tourist path. We can hypothesize that fungal communities at the cave entrance are composed of different species, which can cope well with the new conditions induced by visitors with respect to the assemblages in the deepest parts. On the contrary, fungal communities in the control area are composed of resident species that are expected to be less resilient to human disturbance.

Variation of fungal communities at different cave depths is supported by the observed decline in species replacement at increasing distance from the cave entrance. Based on these results, the composition of species assemblages of Fungi is driven by dispersal, as demonstrated by their higher turnover at the entrance of the cave with respect to the deepest parts. This is in agreement with previous studies demonstrating that Fungi from the external environment can be vehiculated in caves by air circulation (Docampo et al., 2011; Ogórek et al., 2014, 2016).

Environmental filtering plays a significant role in determining the assemblages of Fungi and, to a lesser extent, of Archaea. Both groups showed a decline of their ASV-ratio at increasing dissimilarity of sediment composition from the control areas, together with an increase in species replacement for Fungi. Given that the distance from the tourist path and from the cave entrance did not influence sediment composition, this response can be specifically ascribed to the filter exerted by the environment. By examining the contribution of each physical and chemical parameter to the overall variation of sediment composition, we could identify Nitrogen concentration and, to a lesser extent, pH and the % of clay as the most important variables. We can therefore hypothesize that these parameters filter species based on their ecological requirements. This is in agreement with literature data, which pointed out a key role of substrate as a driver of species richness

and composition of cave assemblages of both Fungi (Cailhol et al., 2020; Kukla et al., 2018) and Archaea (Marques et al., 2017).

Overall, by adopting a metacommunity framework, we could provide new perspectives on the dynamics and patterns of microbial communities under human disturbance. Specifically, our results highlighted that anthropogenic pressure affects all microbial communities but with different effects. Similarly, the three examined groups show differential responses to environmental filtering and dispersal pointing out that a proper understanding of the underlying selective mechanisms require a comprehensive and multi-taxonomic approach.

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CITED LITERATURE

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TABLES

Table 1 – Loadings of each environmental variable for the 5 axes of the PCA.

Variable	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Total_N	0.453	0.320	-0.029	-0.561	0.614
C_org	0.361	0.221	-0.848	0.157	-0.278
pH	-0.473	-0.237	-0.440	0.250	0.681
% Clay	0.448	0.157	0.278	0.737	0.274
% Silt	0.268	-0.687	-0.092	0.210	-0.036
% Sand	-0.410	0.554	-0.027	-0.102	-0.076

Table 2 – Estimated parameters (β-est), standard errors (SE), t-values (t) and p-values (P) for each covariate in the GLMMs performed on: a) ASV-ratios, b) β- replacement, and c) β- richness of the three examined groups. Dist_path = ordinal variable representing increasing distance from the tourist path; Dist_entrance = ordinal variable representing increasing distance from the tourist path; substrate = dissimilarity of the substrate composition from the control area. Significant results are highlighted in bold.

Group	**Variable**	β-est	SE	t	P
ASV-ratios	ASV-ratios	ASV-ratios	ASV-ratios	ASV-ratios	ASV-ratios
Fungi	Dist_path	-1.02	0.405	-2.53	**0.012**
	Dist_entrance	-0.637	0.392	-1.63	0.104

	Substrate	-4.26	1.63	-2.62	**0.009**
	Dist_path:Dist_entrance	0.489	0.179	2.73	**0.006**
Bacteria	Dist_path	-0.237	0.210	-1.13	0.260
	Dist_entrance	-0.137	0.193	-0.708	0.479
	Substrate	0.018	2.43	0.008	0.994
Archaea	Dist_path	0.011	0.115	0.099	0.921
	Dist_entrance	-0.202	0.109	-1.856	0.063
	Substrate	-3.06	1.35	-2.28	**0.023**
β-replacement	β-replacement	β-replacement	β-replacement	β-replacement	β-replacement
Fungi	Dist_path	-0.250	0.220	-1.14	0.255
	Dist_entrance	-0.398	0.208	-1.92	0.056
	Substrate	6.50	2.39	2.72	**0.007**
Bacteria	Dist_path	-0.509	0.225	-2.26	**0.024**
	Dist_entrance	-0.377	0.215	-1.75	0.080
	Substrate	-1.06	2.29	-0.461	0.645
Archaea	Dist_path	-0.727	0.259	-2.80	**0.005**
	Dist_entrance	-0.395	0.214	-1.85	0.065
	Substrate	-3.16	2.24	-1.41	0.159
β-richness	β-richness	β-richness	β-richness	β-richness	β-richness
Fungi	Dist_path	0.219	0.236	0.925	0.355
	Dist_entrance	0.295	0.223	1.33	0.185
	Substrate	-6.77	2.65	-2.56	**0.011**
Bacteria	Dist_path	0.429	0.240	1.79	0.074
	Dist_entrance	0.232	0.232	0.998	0.318
	Substrate	0.381	2.53	0.151	0.880
Archaea	Dist_path	0.737	0.269	2.73	**0.006**
	Dist_entrance	0.273	0.233	1.17	0.241
	Substrate	2.58	2.45	1.05	0.293