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Does topography influence spatial variation in microbial communities along mountain bike trails to a greater degree than trail use itself?

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Abstract

In this study, we evaluated the impact of mountain biking on microorganisms along forest trails. We conducted environmental DNA sampling and biodiversity analyses using swabbing to collect surface particulate matter from mountain bike trails in the mountainous region of Shizuoka Prefecture. The study design involved sampling at the middle of the trail, near the beginning and end of the trail, and in a broad-leaved forest completely outside the trail, to enable a comparison of areas with and without the influence of mountain biking. Analysis of environmental DNA from prokaryotes and eukaryotic microorganisms (primarily fungi) revealed that on mountain bike trails, microbial communities in the trail center—which is highly susceptible to the impact of biking—and at both ends of the trail—which are relatively less affected—showed similarities not only to each other, but also to surface microorganisms in the broadleaf forest area, indicating that overall microbial diversity is maintained. On the other hand, significant changes in microbial communities were detected at specific locations, suggesting that topographical factors have a greater influence on microbial community dynamics than mountain biking. In the future, we expect that this methodology will facilitate assessment of the impact of human activities on microbial communities not only on mountain bike trails, but also on hiking and horseback riding trails, and that it will be used to evaluate microbial ecosystems with a focus on "nature-positive" principles.

Introduction

Trails provide recreational opportunities to connect with nature through activities such as hiking and mountain biking. Given that there are concerns about the impact of such human activities on the environment[1], restricting human activities to designated trails may promote environmental conservation [2]. Several previous studies have examined impacts of walking and mountain biking on the environment surrounding trails. The review by Pickering et al. (2010) presented various environmental assessments regarding the impacts of hiking, horseback riding, and mountain biking. Among these, potential impacts on vegetation, biomass, and microorganisms are discussed [3]. Nonetheless, to date, specific information regarding impacts of such activities on microbial communities is extremely limited.

Soil microorganisms are crucial for viability of forest communities, including

succession and coexistence[4]. In forest soils, microorganisms drive the carbon and nitrogen cycles through litter decomposition and nutrient mineralization, and are key drivers of biochemical cycling in forest ecosystems[5, 6, 7]. Fungi, in particular, are involved in decomposition of cellulosic matter, such as lignin and lignocellulose, and promote cycling of carbon and nutrients derived from plant litter [8, 9]. Furthermore, soil microorganisms are involved not only in geochemical cycling, but also in the structural formation of forest tree communities. Bennett et al. (2017) demonstrated that in arbuscular mycorrhizal trees, seedling growth is inhibited in the soil beneath mature trees of the same species, whereas in ectomycorrhizal trees, growth of conspecific seedlings is enhanced. This suggests that plant–soil feedback mediated by mycorrhizal type may influence forest population dynamics and community structure [10]. Thus, forest soil microorganisms serve as important biological indicators, reflecting ecosystem functions, geochemical cycles, and community structure, and are an indispensable element in forests.

Kadowaki et al. (2023) demonstrated the usefulness of microbial diversity as a soil indicator, demonstrating changes in microbial communities associated with shifts in vegetation caused by deer browsing. Therefore, assessing microbial communities along trails provides an opportunity to understand how trail use potentially affects biodiversity. Environmental DNA (eDNA) methods, which have gained prominence in recent years, involve directly sequencing and analyzing biological DNA from environmental samples such as water and soil[11] and are attracting attention for assessing biodiversity. While there is a wealth of knowledge regarding environmental DNA analysis targeting soil microorganisms in terrestrial environments [12, 13], to the best of our knowledge, no studies have evaluated microbial diversity through environmental DNA analysis in locations such as trails. Although swabbing with cotton swabs yields only limited sample quantities, its simplicity makes it applicable in various terrestrial locations. Furthermore, because it is easy to increase the number of replicates, it is advantageous for designing study protocols to compare impacts of human activities, such as hiking and mountain biking.

In this study, to clarify the impact of mountain biking on microbial communities along a mountain bike trail in a broad-leaved forest in Morimachi, Shizuoka Prefecture, we

focused on the middle of the trail, where the impact of mountain biking is likely to be most pronounced and at both ends, where the impact is likely to be minimal. We compared these sites with an undisturbed area of broadleaf forest to assess spatial variation in microbial communities along the entire trail. With regard to microorganisms, in addition to prokaryotic communities, we also focused on eukaryotic communities such as fungi. By simultaneously conducting community analysis at different taxonomic levels, we sought to comprehensively characterize diverse microbial communities.

Materials and Methods

Sampling sites

Sampling was conducted on November 17, 2024, on the trail at Million Petal Bike Park, located in Mikura, Morimachi, Shuchi District, Shizuoka Prefecture. In the vicinity of the study site, annual precipitation amounts to roughly 2,425 mm with an annual average temperature of 16.1°C, indicating a warm, rainy environment. Annual precipitation was based on the 1991–2020 values from the Japan Meteorological Agency's Mikura Regional Rainfall Observatory, and the annual average temperature was based on the 1991–2020 values from the Japan Meteorological Agency's Tenryu Regional Meteorological Observatory.

Sampling and Molecular Experiment

Six sampling transects were established: Transects 1 and 2 at the start of the trail, Transects 3 and 4 near the turnaround point, and Transects 5 and 6 near the section with a continuous slope (Figure 1). Transects 1 and 2, located near the starting point, are at relatively higher elevation, whereas Transects 5 and 6 are located at lower elevation. For each transect, three sampling sites were designated (one at the center of the trail and two at its sides). Additionally, two sampling points were established in a nearby broadleaf forest lateral to the trail. At each location, surface swabbing was performed using sterile cotton swabs (HUBY-COTIX standard, Sanyo Corporation, Osaka, Japan). Four samples were collected at each location to allow comparison of the presence or absence of mountain bike activity (Table S1). During sampling, gloves were worn, and the scissors used to cut the swabs were cleaned with hypochlorous acid to eliminate contaminating DNA.

Swab heads were soaked in Buffer ATL and disrupted using MN Bead Tubers Type A and a Shake Master (Biomedical Science, Tokyo, Japan). DNA extraction from the disrupted samples was performed using a DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) in accordance with the product manual. Additionally, to prevent contamination, DNA extraction was performed following the environmental DNA protocol described by Minamoto et al. (2021). For prokaryotes, the V3–V4 variable regions of the 16S rRNA gene were amplified using forward and reverse primers (P341F-P805R[14]) containing adapter sequences. For fungi, the variable region of the internal transcribed spacer 1 (ITS1) was amplified using a forward primer (ITS5[15]) and a reverse primer (ITS2-KYO2[16]), both containing adapter sequences. PCR amplification and library preparation were performed following the same procedures as in our previous study [17]. Sequencing was performed using a MiSeq sequencer (Illumina, San Diego, California, USA). For sequencing the 16S rRNA and ITS1 regions, paired-end sequencing (2×300 bp) was conducted according to the MiSeq Reagent Kit V3 (Illumina). Raw data from these assays have been submitted to the DNA Data Bank of Japan (DDBJ) and are available under accession numbers DRR1068750–DRR1068829 (16S rRNA analysis) and DRR1068673–DRR1068749 (ITS1 analysis).

Bioinformatics

For 16S rRNA and ITS1 sequences, we analyzed the FASTQ files obtained from the above experiments using the QIIME2 platform [18]. Low-quality reads were excluded, and chimeric reads were identified and removed using "dada2." For the reference databases, 16S rRNA sequences were clustered into amplicon sequence variants (ASVs) based on matches to SILVA (silva-132-99-nb-classifier.qza[19]), and ITS1 sequences were clustered into ASVs based on matches to UNITE (unite_ver10_97[20]). Using ASV matrix data obtained from QIIME2, we performed further processing in R[21]. For the 16S rRNA ASV matrix, Eukaryota, Mitochondria, Chloroplast, and Unassigned were removed. Using the "vegan" package[22], coverage-based rarefaction was applied to read counts for each ASV [23]. ASVs with a frequency of 0.1% or less in each sample were removed. Using the transformed ASV matrix, the "vegan" package was used to calculate the species richness index (Shannon-Weaver index) for each site, and the significance of differences in mean values was verified using Welch's t-test. Using the transformed

ASV matrix, the software package “pvclust” [24]was employed to perform correlation analysis and hierarchical cluster analysis using Ward’s method. Classified clusters were statistically evaluated by determining the multiscale bootstrap probability (AU) and the general bootstrap probability (bootstrap P-value: BP) based on 1,000 bootstrap resamples. We performed non-metric multidimensional scaling (nMDS) based on Bray-Curtis distance using the “vegan” package. We conducted non-hierarchical cluster analysis using the K-medoids method with “cluster” v. 2.1.3 [25] and estimated the number of clusters using silhouette values. Results of the nMDS and non-hierarchical cluster analysis were visualized using the “ggplot2” package [26]. Additionally, indicator values were calculated using the indval function from R’s “labdsv” package [27] to detect characteristic taxa associated with specified clusters, and indicator species analysis was performed. Analysis of variance (ANOVA) for diversity indices (number of species and Shannon-Weaver index) was performed using the Type II option of the “Anova“ function in the R “car“ package. Multiple comparisons were performed using the “pairs“ function (with adjust = “tukey”) in the “emmeans“ package.

Results

As a result of molecular analysis targeting the 16S rRNA region of prokaryotes, sequence data were obtained from all 80 samples collected from Transects 1–6 and from under broadleaf trees. At the L5 (family) level in the QIIME2 analysis, 277 taxonomic groups were identified after filtering, with Acidobacteriaceae, Solibacteraceae, Mycobacteriaceae, and Acidothermaceae being the predominant groups (Fig. 2). In the molecular analysis targeting the ITS1 region in fungi, sequencing data were obtained from 76 samples—excluding four samples (Sample IDs: 22, 74, 75, and 77)—collected from Transects 1–6 and under broadleaf trees. At the L5 (family) level of the QIIME2 analysis, 228 taxonomic groups were identified after filtering, with Mytilinidiales, Cucurbitariaceae, Sympoventuriaceae, Herpotrichiellaceae, and Trichomeriaceae being the major groups (Fig. 3).

In the β-diversity analysis using the ASV table derived from 16S rRNA metabarcoding, the maximum silhouette value was 3, a result supported by the hierarchical cluster analysis (Fig. S1). Among these, Cluster 3, centered on Transect 5, was particularly prominent (Fig. 4 and Table S2); however, no tendency was observed for microbial

communities to be differentiated between the center and edges of the trail or between those on the trail and those under broadleaf trees. Cluster 1 tended to be concentrated in Transects 1 and 2 (Table S2). In the indicator species analysis, 39 taxa were detected in Cluster 1, 27 in Cluster 2, and 32 in Cluster 3 (Table S3). Diversity indices tended to be lower in Transect 5 (Fig. 4). In the analysis of variance for species richness and the Shannon-Weaver index, significant differences were observed between the categories of each transect and those under broadleaf trees (data for Tree 1 and Tree 2 were pooled due to their small sample sizes) ($p < 0.01$). Furthermore, results from multiple comparisons showed significant differences in all pairings between Transect 5 and the other categories ($p < 0.01$), but no significant differences were observed in the other pairings.

Analysis using the ASV table from ITS1 metabarcoding showed that the maximum silhouette value was 2, a finding supported by results of hierarchical cluster analysis (Fig. S2). Among these, a cluster centered on Transects 5 and 6 was particularly prominent (Fig. 5 and Table S4); however, no tendency was observed for microbial communities to diverge between the center and edges of the trail or laterally in the forest. In the indicator species analysis, 10 taxa were detected in Cluster 1 and 10 in Cluster 2 (Table S5). Diversity indices tended to be lower at Transect 5 (Fig. 5). In the analysis of variance for species richness and the Shannon-Weaver index, species richness was significantly different between the categories of each transect and the broadleaf tree canopy (data for Tree 1 and Tree 2 were pooled due to their small sample sizes) ($p < 0.01$), but no significant difference was observed for the Shannon-Weaver index ($p = 0.08$). Furthermore, in the results of multiple comparisons of species richness, significant differences were observed in all pairings between Transect 5 and the other categories except Tree ($p < 0.01$), but no significant differences were found in the other pairings.

Discussion

Overview of microbial community structure across the entire trail

In this study, by employing a swabbing sampling method, we were able to characterize microbial communities across a large mountain bike trail while using a high number of replicates (four replicates per site). In eDNA metabarcoding analysis, it has been

pointed out that ensuring biological replicates is crucial for achieving reliable species detection. That is, analyzing multiple samples from a single site yields a higher expected number of detected species than analyzing a single sample[28]. In this study, although samples consisted of fine particles from the topsoil, we endeavored to ensure a high degree of biological replication by collecting four samples from each site for analysis.

An overview of the microbial community structures of prokaryotes and fungi detected through eDNA metabarcoding analysis revealed that along the mountain bike trail, microbial composition was similar not only between the more vulnerable central position of the trail and the relatively less vulnerable trail edges, but also when compared to surface-layer microorganisms in undisturbed broadleaf forest areas, indicating that the microbial composition was similar overall. On the other hand, marked differences in microbial communities were detected at a specific location (Transect 5) for both prokaryotic and fungal communities. Pickering et al. (2011) reported that mountain biking causes more severe impacts than hiking, whereas Chiu and Kriwoken (2003) found no significant difference in soil loss between mountain bike and hiking trails. This contradiction suggests that different management and use-related factors may be involved in degradation of hiking and cycling trails [2]. Since Transects 5 and 6 were relatively newly constructed steep slopes, they may have been formed at a different time than the other transects, and the degree of management observed locally along the trail may have led to heterogeneity in microbial communities.

Details of prokaryotic microbial community structure

Of the three clusters detected in the prokaryotic microbial community, Cluster 1 showed a tendency to be biased toward Transects 1 and 2 (Table S2). In the analysis of indicator species for the prokaryotic community, Cluster 1 included Rhizobiales [31], which comprises major groups of plant rhizosphere microorganisms, Bacteroidota [32], which includes groups involved in degradation of organic matter, such as polysaccharides. Actinobacteria [33], and Sphingomonadaceae [34] were identified as indicator taxa in Cluster 1. This indicates an environment in which active organic matter degradation occurs due to litter input, while also being influenced by the plant rhizosphere. Shigyo et al. (2022) showed that observed soil bacterial taxa and metabolic function indicators

254 varied significantly depending on the location on the slope. Among various trail
255 management-related factors, topography has been identified as the most critical factor
256 for trail sustainability [2]. Transects 1 and 2, established relatively earlier than the other
257 lines, are located at the start of the trail, and are at a relatively higher elevation than
258 other locations. These trail construction processes along the trail and local topographic
259 factors may influence dynamics of microbial communities characterized by Cluster 1.
260
261 Cluster 2 of the prokaryotic microbial community was observed throughout the trail,
262 and groups reportedly adapted to acidic conditions, such as the Acidobacteriales, which
263 includes the Rhodanobacteraceae and Acidobacteriaceae subgroup 1, were strongly
264 detected as indicators [35, 36], suggesting that the soil environment is predominantly
265 acidic. In addition to these groups, those such as Mycobacteriaceae, which have been
266 reported to degrade resistant organic compounds such as polycyclic aromatic
267 hydrocarbons (PAHs) [37], and groups such as WPS-2, which are thought to be adapted to
268 stressful environments, such as low-nutrient, arid, and acidic conditions [38], have also
269 been detected as indicator species. Thus, Cluster 2 is composed of groups that slowly
270 degrade organic matter under acidic and low-nutrient conditions. These characteristics
271 of the microbial community are considered representative of the entire trail, which is
272 located in a forest.
273
274 Cluster 3 is largely localized to Transect 5. Bathyarchaeia, which exhibited the highest
275 indicator value among the prokaryotic microbial communities in Cluster 3, is found in
276 hypoxic environments and is involved in processes such as anaerobic organic
277 decomposition [6, 39]. Furthermore, while Methanomassiliicoccales, which is involved in
278 methylotrophic methanogenesis under anaerobic conditions, was detected as an
279 indicator species [40, 41]. Nitrososphaeria, Nitrosotaleaceae, and Nitrospiraceae, which are
280 involved in aerobic nitrification, including ammonia oxidation, nitrite oxidation, or
281 complete ammonia oxidation,were also detected as indicator species [42, 43, 44]. Soils with
282 such spatially distributed microenvironments of differing oxidative and reductive
283 conditions are thought to result from poor drainage and insufficient oxygen supply [45], as
284 well as from oxygen consumption due to microbial respiration and oxygen diffusion
285 limitations occurring in microbial hotspots formed by localized accumulation of litter
286 and organic matter [46]. Such redox heterogeneity, a characteristic of the environment

along Transect 5, which was developed relatively recently and is located at a lower elevation along the trail, may have influenced the formation of microbial communities detected in this study.

Overview of fungal microbial community structure

Of the two clusters detected in the fungal microbial community, the Pestalotiopsidaceae and Diaporthales, identified as indicator taxa in Cluster 1, contain many endophytes, pathogens, and saprophytes associated with plant tissues [47, 48], while Xylariales and Polyporales are reportedly involved in decomposition of lignocellulosic substrates and plant residues [49, 50]. Furthermore, since the Chaetothyriales includes black fungi, which exhibit tolerance to environmental stresses such as drought, ultraviolet radiation, and nutrient deficiency [51, 52], Cluster 1 may reflect fungal communities involved in decomposition of fresh litter and dead plant fragments supplied to the forest soil surface, as well as those responding to physical and drought stresses in the surface environment. Cluster 2 was concentrated at Transects 5 and 6, with the families Russulaceae and Cortinariaceae, which contain many ectomycorrhizal fungi, showing high values as indicator species. These fungal groups are involved in acquisition of organic nitrogen, such as amino acids, proteins, and chitin, as well as in enzymatic conversion of soil organic matter that is difficult to decompose, such as humus and phenolic organic compounds. They may therefore be involved in metabolism of slow-decomposing organic matter and organic nitrogen in Transects 5 and 6 [53, 54, 55, 56, 57].

Future directions

Regarding the impact of mountain biking and hiking on trail expansion and deepening, it has been shown that while relatively limited, this impact is influenced by environmental conditions[1]. In the future, to gain a comprehensive understanding of microbial community diversity on trails, it will be necessary to track seasonal changes in microbial communities to observe how similarities and differences in communities observed in this study change. Furthermore, since it has been noted that microbial composition varies with soil depth [58], it is also necessary to take into account changes in diversity along the vertical axis. The use of swabbing techniques will facilitate assessment of the impact on microbial communities not only on mountain bike trails, but also on hiking and horseback riding trails in a simple manner. In recent years, as

corporate initiatives toward “nature positive” have garnered significant attention in the environmental sector, proposals have been made to allocate a portion of “nature positive” financing toward assessment of microbial ecosystems [59]. It is hoped that the swabbing-based microbial diversity assessment method implemented on this mountain bike trail will also be utilized in this context.

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Declaration of competing interest

All authors declare that they have no competing interests.

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Figure Legends

Figure 1. A: Map showing the survey area in Million Petal Bike Park and a summary of survey sites. B: Overview of sampling locations. Six transects were established: two lines (Transect 1 and Transect 2) at the beginning of the trail, two transects (3 and 4) near the turnaround point, and two transects (5 and 6) near a section of continuous incline. At each transect, three sampling points were designated: one at the center of the trail and two at its edges (left and right sides). In addition, two locations were selected in the nearby broadleaf forest off the trail (Tree 1 and Tree 2).

Figure 2. A bar plot at the family level for prokaryotes.

Figure 3. A bar plot at the family level for fungi.

Figure 4. Left: nMDS plot of community similarities of prokaryotes. Groups are based on results of cluster analysis setting 3 clusters. Right: diversity index of prokaryotes. TR: Transect, T: Tree (data for Tree 1 and Tree 2 were pooled).

Figure 5. Left: nMDS plot of community similarities of fungi. Groups are based on the results of cluster analysis setting 2 clusters. Right: diversity index of fungi. TR: Transect, T: Tree (data for Tree 1 and Tree 2 were pooled).

Figure S1. Hierarchical cluster analysis of prokaryotes. au: Approximately Unbiased p-value, bp: Bootstrap Probability value.

Figure S2. Hierarchical cluster analysis of fungi. au: Approximately Unbiased p-value, bp: Bootstrap Probability value.

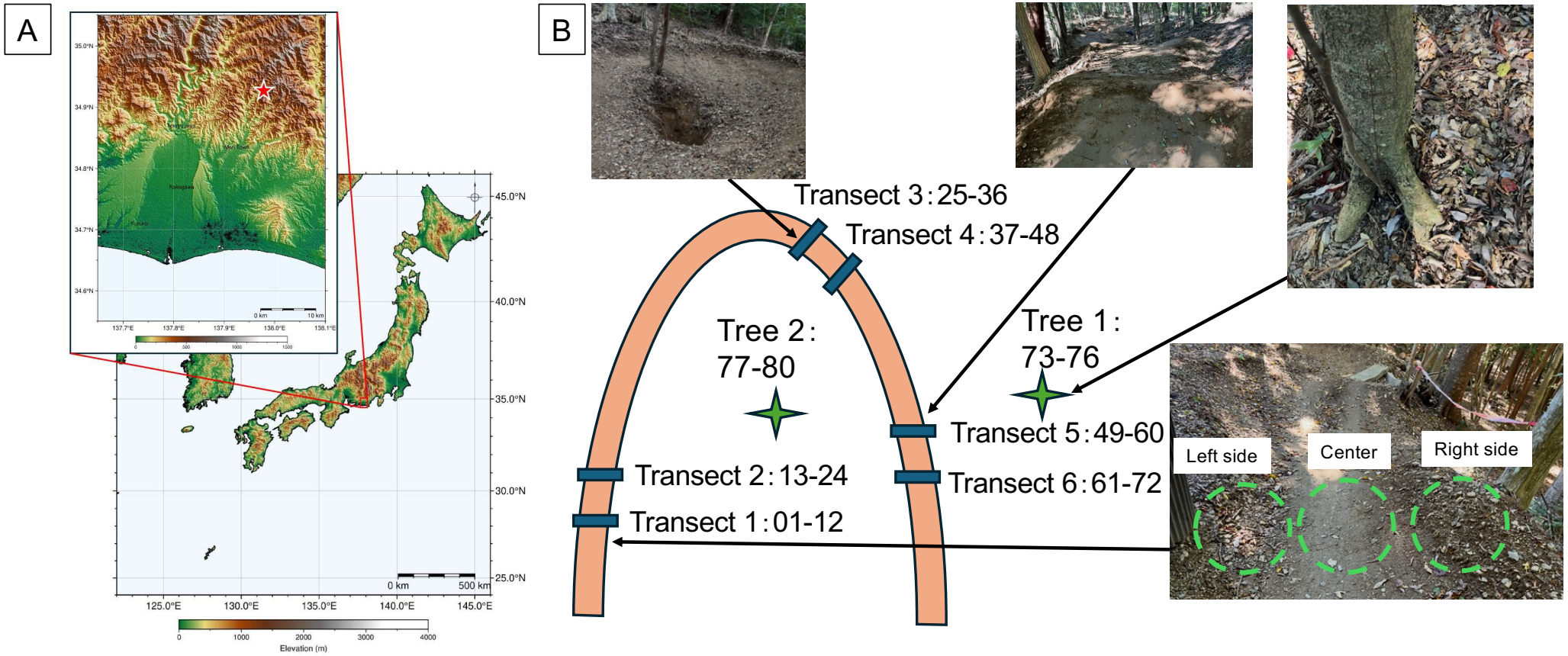

A
B
Transect 3 : 25-36
Transect 4 : 37-48
Tree 2 :
77-80
Tree 1 :
73-76
Transect 5 : 49-60
Transect 2 : 13-24
Transect 6 : 61-72
Transect 1 : 01-12
Left side
Center
Right side
45.0°N
40.0°N
35.0°N
30.0°N
25.0°N
125.0°E
130.0°E
135.0°E
140.0°E
145.0°E
0 km
500 km
0
1000
2000
3000
4000
Elevation (m)

Figure 1

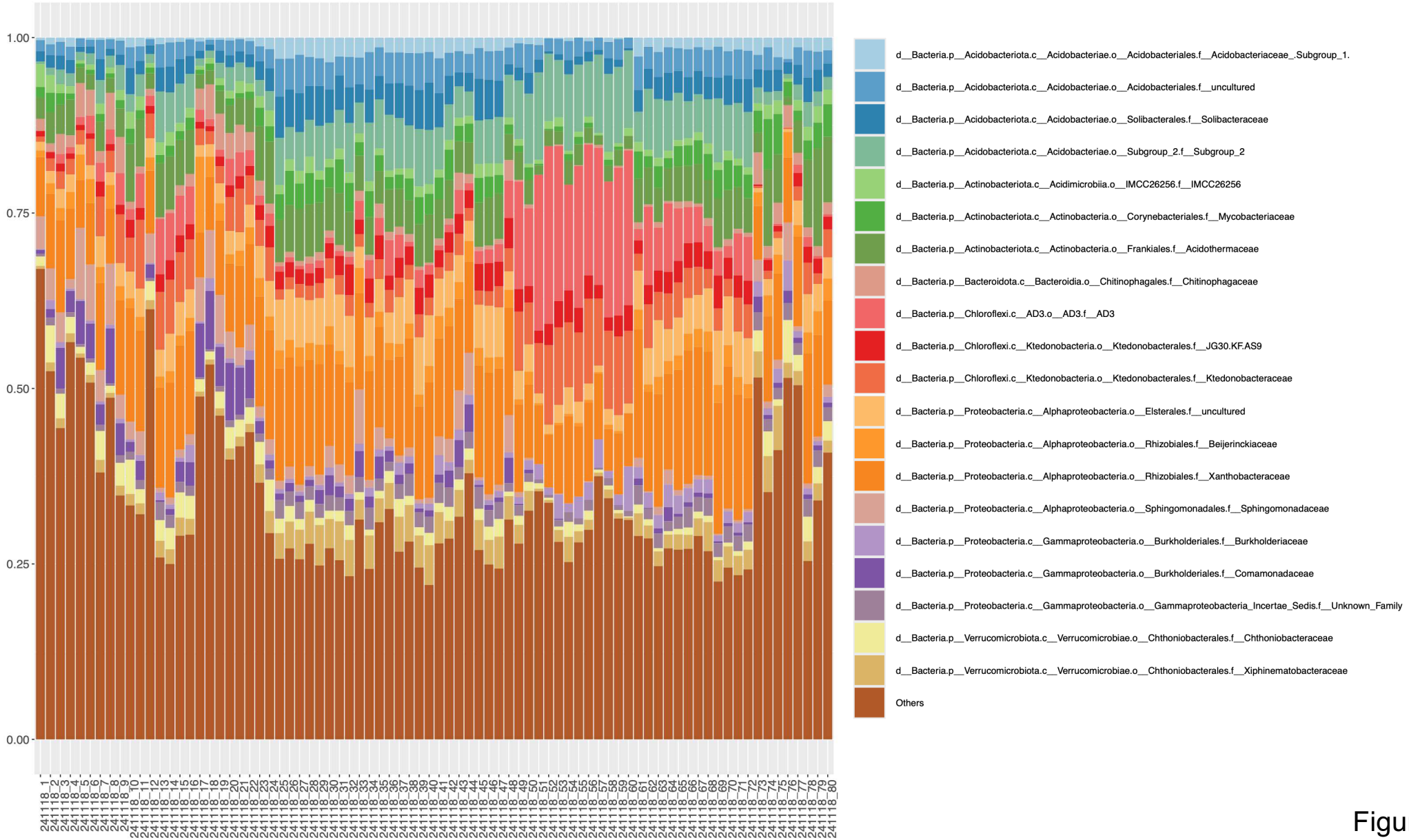

1.00
0.75
0.50
0.25
0.00
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d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Acidobacteriales.f__uncultured
d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Solibacterales.f__Solibacteraceae
d__Bacteria.p__Acidobacteriota.c__Acidobacteriae.o__Subgroup_2.f__Subgroup_2
d__Bacteria.p__Actinobacteriota.c__Acidimicrobiia.o__IMCC26256.f__IMCC26256
d__Bacteria.p__Actinobacteriota.c__Actinobacteria.o__Corynebacteriales.f__Mycobacteriaceae
d__Bacteria.p__Actinobacteriota.c__Actinobacteria.o__Frankiales.f__Acidothermaceae
d__Bacteria.p__Bacteroidota.c__Bacteroidia.o__Chitinophagales.f__Chitinophagaceae
d__Bacteria.p__Chloroflexi.c__AD3.o__AD3.f__AD3
d__Bacteria.p__Chloroflexi.c__Ktedonobacteria.o__Ktedonobacterales.f__JG30.KF.AS9
d__Bacteria.p__Chloroflexi.c__Ktedonobacteria.o__Ktedonobacterales.f__Ktedonobacteraceae
d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Elsterales.f__uncultured
d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Beijerinckiaceae
d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Rhizobiales.f__Xanthobacteraceae
d__Bacteria.p__Proteobacteria.c__Alphaproteobacteria.o__Sphingomonadales.f__Sphingomonadaceae
d__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Burkholderiales.f__Burkholderiaceae
d__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Burkholderiales.f__Comamonadaceae
d__Bacteria.p__Proteobacteria.c__Gammaproteobacteria.o__Gammaproteobacteria_Incertae_Sedis.f__Unknown_Family
d__Bacteria.p__Verrucomicrobiota.c__Verrucomicrobiae.o__Chthoniobacterales.f__Chthoniobacteraceae
d__Bacteria.p__Verrucomicrobiota.c__Verrucomicrobiae.o__Chthoniobacterales.f__Xiphinematobacteraceae
Others

Figure 2

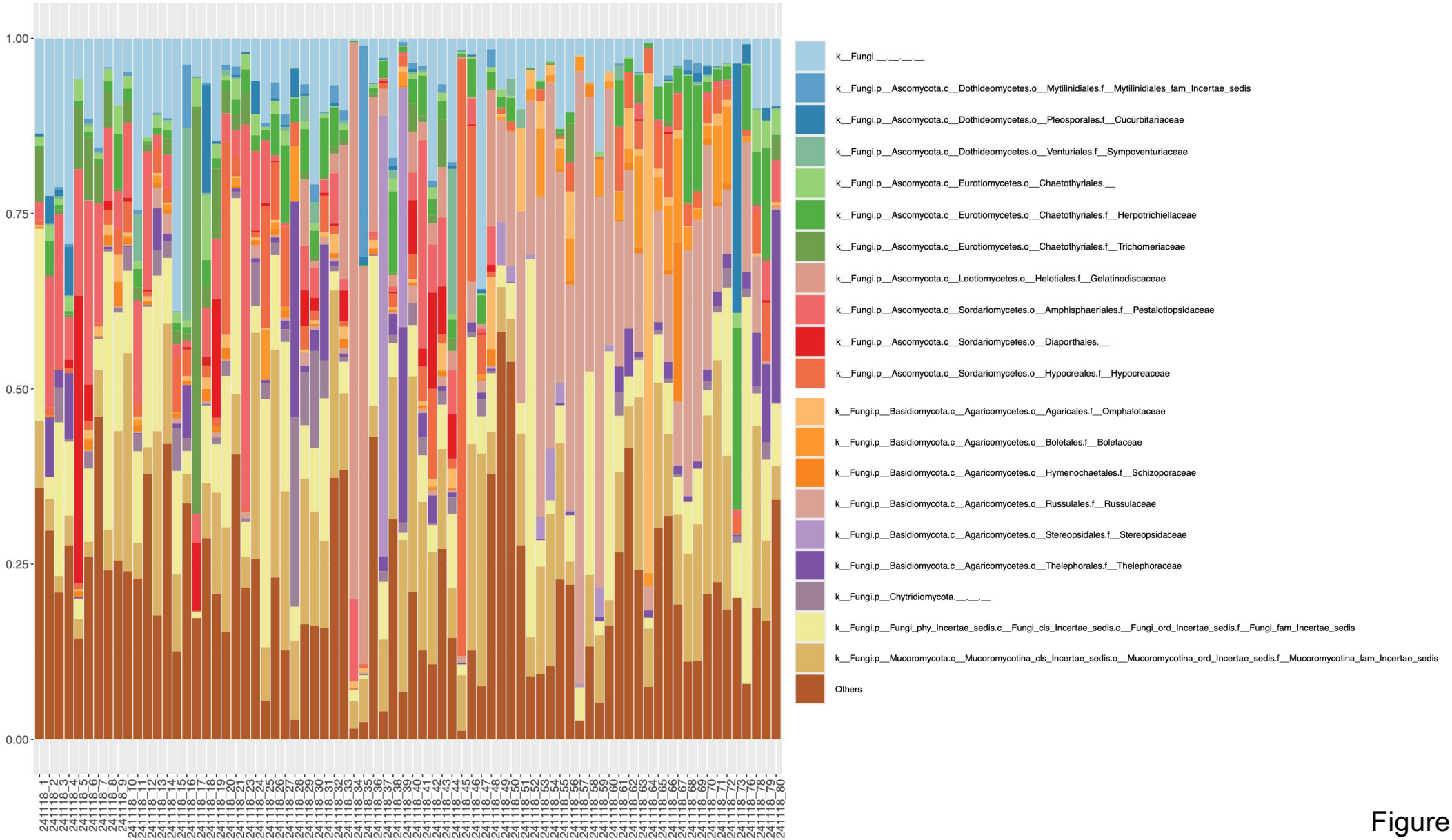
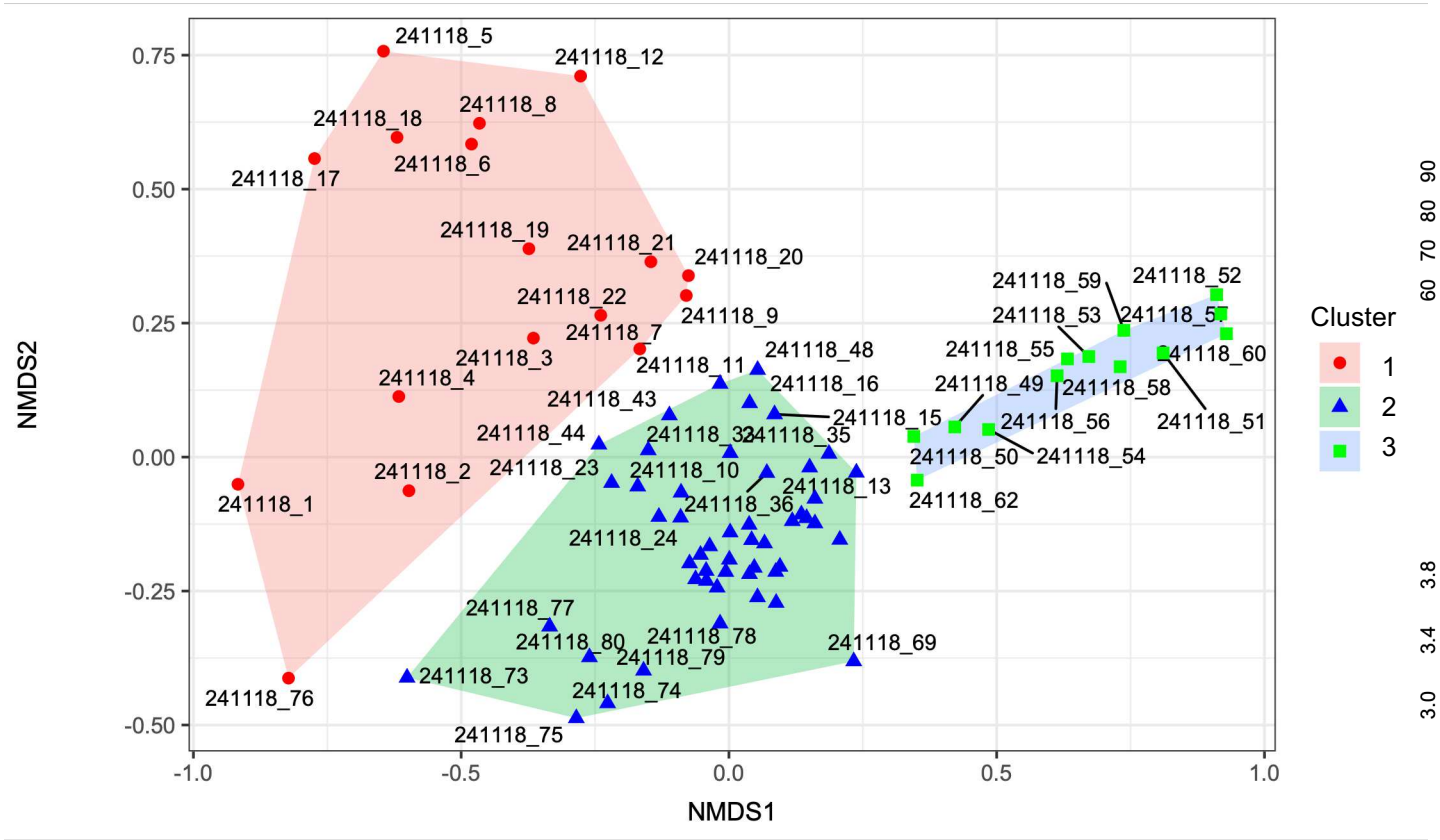

1.00
0.75
0.50
0.25
0.00
k__Fungi.__.__.__.__
k__Fungi.p__Ascomycota.c__Dothideomycetes.o__Mytilinidiales.f__Mytilinidiales_fam_Incertae_sedis
k__Fungi.p__Ascomycota.c__Dothideomycetes.o__Pleosporales.f__Cucurbitariaceae
k__Fungi.p__Ascomycota.c__Dothideomycetes.o__Venturiales.f__Sympoventuriaceae
k__Fungi.p__Ascomycota.c__Eurotiomycetes.o__Chaetothyriales.__
k__Fungi.p__Ascomycota.c__Eurotiomycetes.o__Chaetothyriales.f__Herpotrichiellaceae
k__Fungi.p__Ascomycota.c__Eurotiomycetes.o__Chaetothyriales.f__Trichomeriaceae
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k__Fungi.p__Mucoromycota.c__Mucoromycotina_cls_Incertae_sedis.o__Mucoromycotina_ord_Incertae_sedis.f__Mucoromycotina_fam_Incertae_sedis
Others

Figure 3


241118_5
241118_12
241118_8
241118_18
241118_6
241118_17
241118_19
241118_21
241118_20
241118_22
241118_9
241118_7
241118_3
241118_11
241118_48
241118_4
241118_16
241118_43
241118_15
241118_44
241118_32
241118_35
241118_2
241118_23
241118_10
241118_13
241118_1
241118_36
241118_24
241118_77
241118_80
241118_78
241118_79
241118_69
241118_73
241118_74
241118_76
241118_75
241118_59
241118_52
241118_53
241118_55
241118_49
241118_58
241118_60
241118_56
241118_51
241118_50
241118_54
241118_62
0.75
0.50
0.25
0.00
-0.25
-0.50
-1.0
-0.5
0.0
0.5
1.0
NMDS1
NMDS2
Cluster
1
2
3

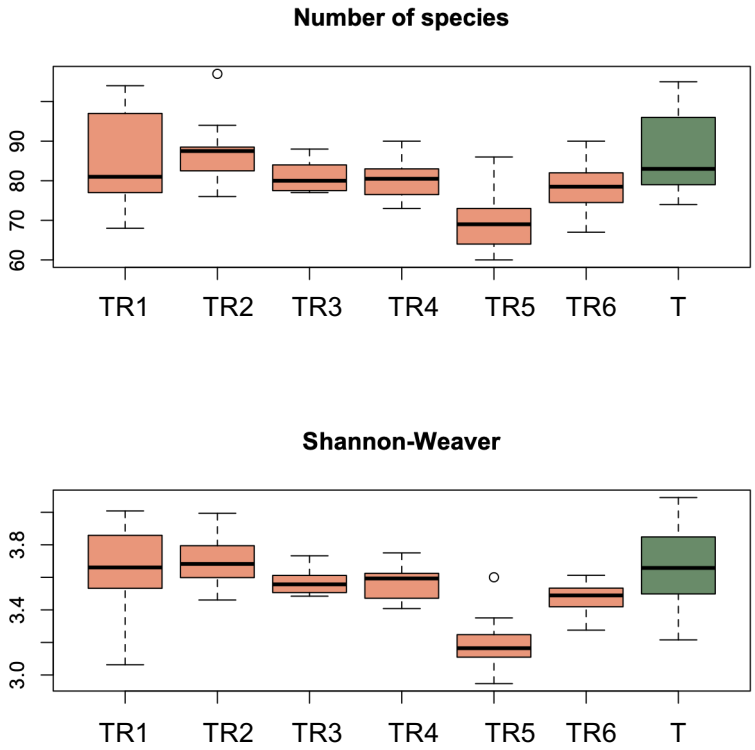
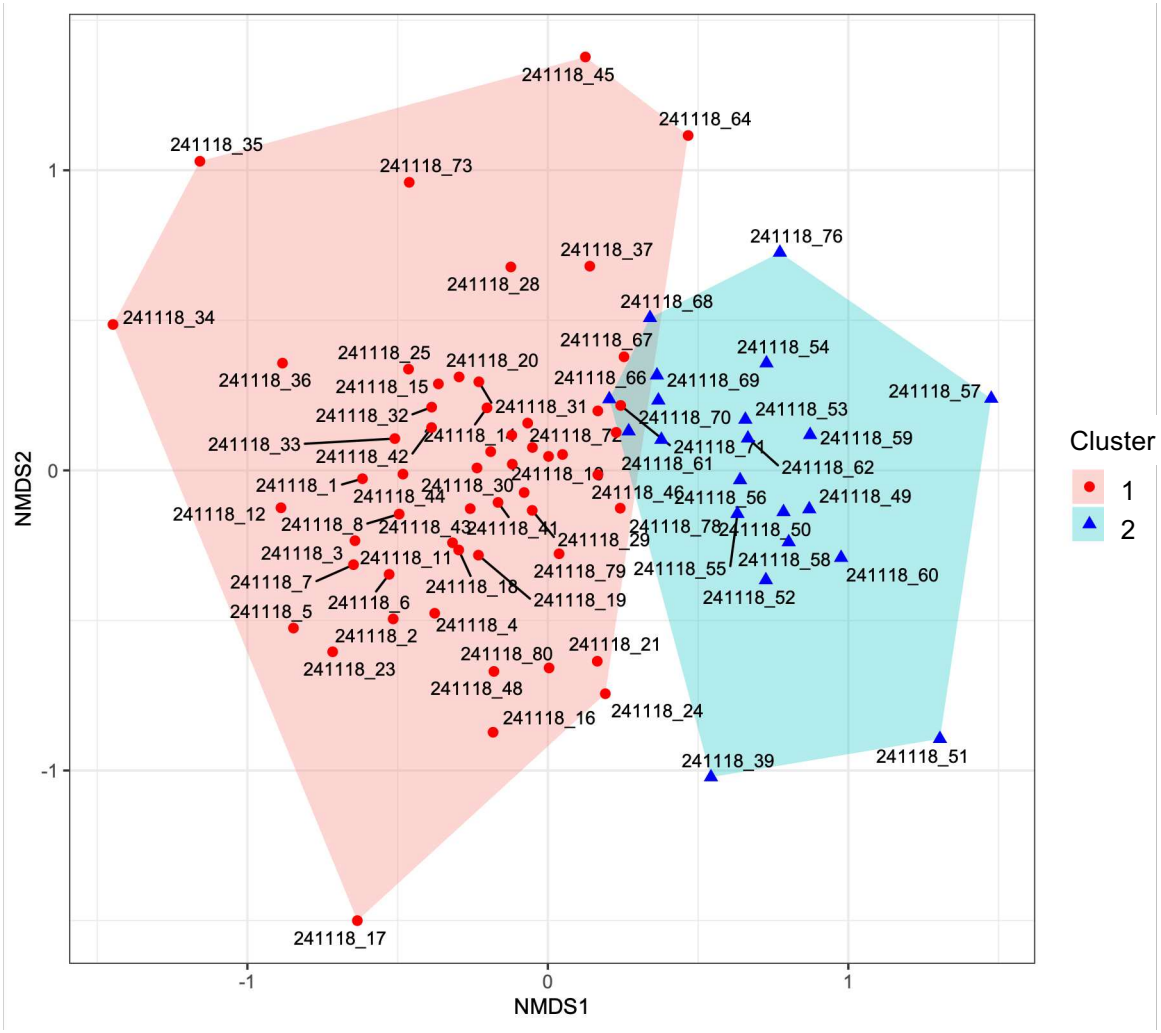
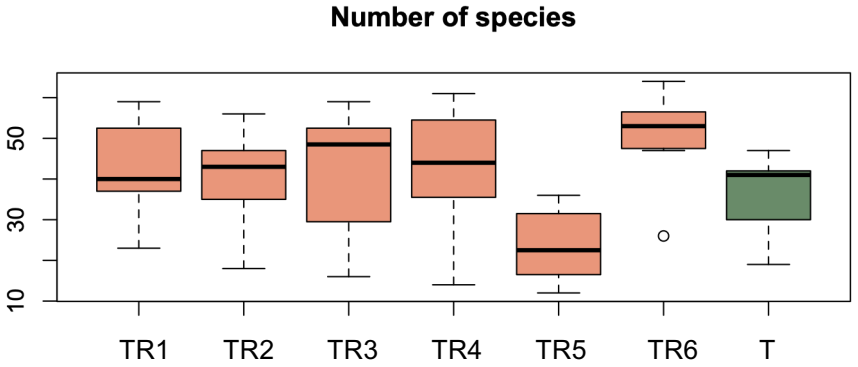

Number of species
60
70
80
90
TR1
TR2
TR3
TR4
TR5
TR6
T
Shannon-Weaver
3.0
3.4
3.8
TR1
TR2
TR3
TR4
TR5
TR6
T

Figure 4


NMDS1
NMDS2
Cluster
1
2
241118_45
241118_64
241118_35
241118_73
241118_76
241118_37
241118_28
241118_68
241118_34
241118_67
241118_54
241118_25
241118_20
241118_36
241118_15
241118_66
241118_69
241118_57
241118_32
241118_31
241118_70
241118_53
241118_33
241118_14
241118_72
241118_59
241118_42
241118_71
241118_61
241118_62
241118_1
241118_10
241118_44
241118_30
241118_46
241118_56
241118_49
241118_12
241118_8
241118_43
241118_41
241118_78
241118_50
241118_29
241118_3
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241118_79
241118_55
241118_58
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241118_7
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241118_19
241118_52
241118_5
241118_6
241118_4
241118_2
241118_21
241118_23
241118_80
241118_48
241118_24
241118_16
241118_39
241118_51
241118_17


Number of species
TR1
TR2
TR3
TR4
TR5
TR6
T

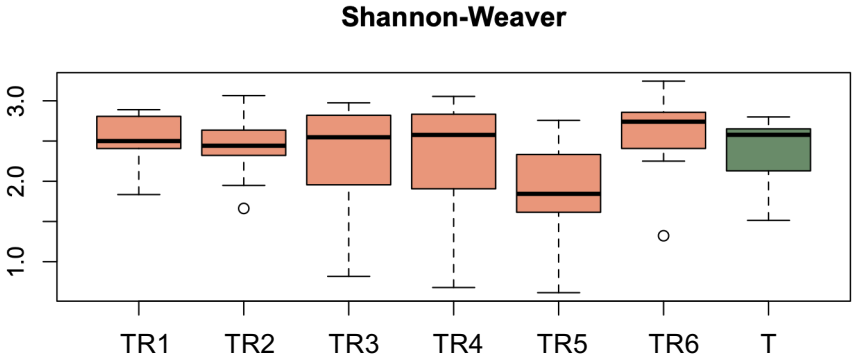

Shannon-Weaver
TR1
TR2
TR3
TR4
TR5
TR6
T

Figure 5

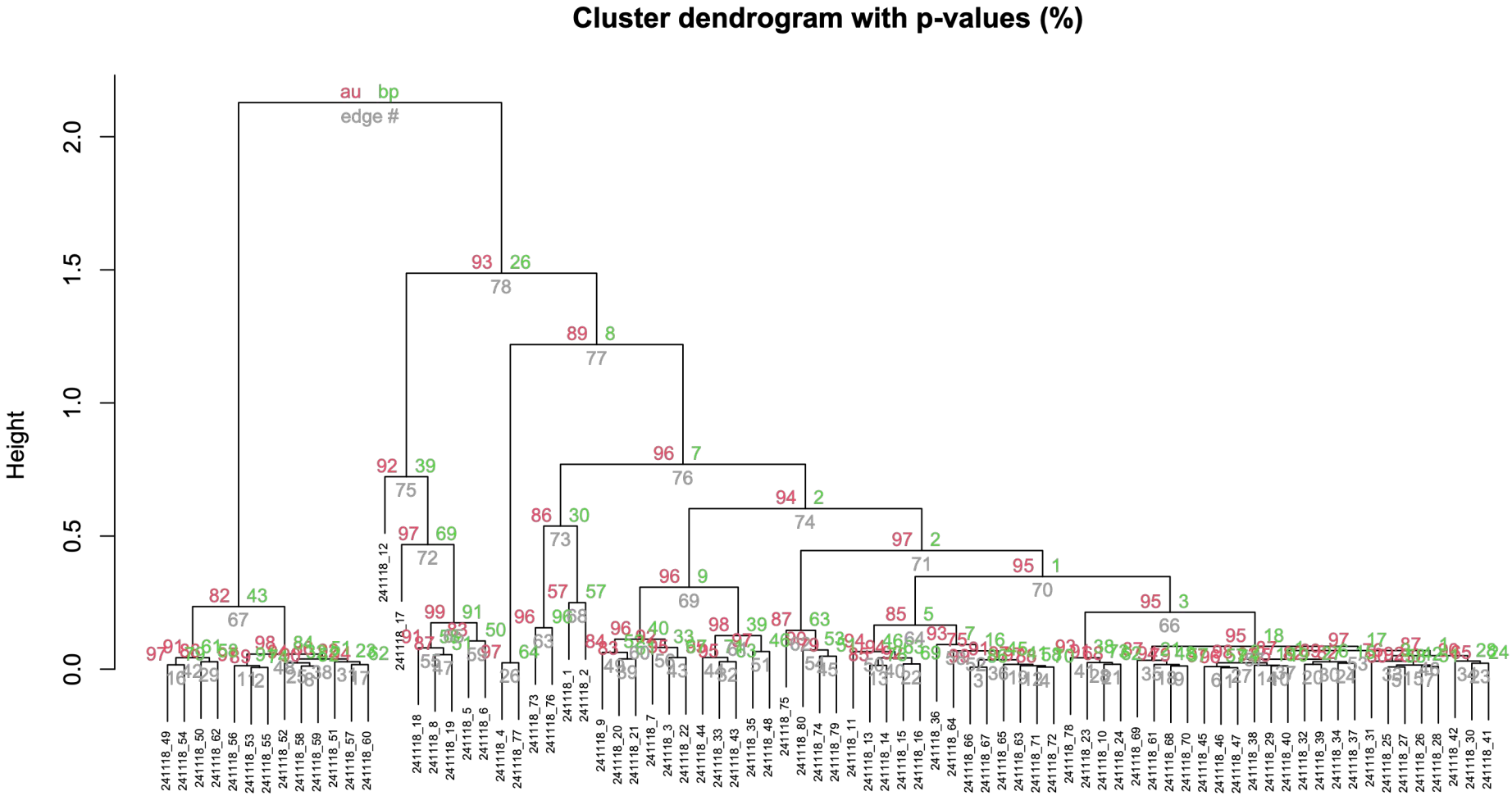

Cluster dendrogram with p-values (%)
au bp
edge #
Height
0.0
0.5
1.0
1.5
2.0
Distance: correlation
Cluster method: ward.D2

Figure S1

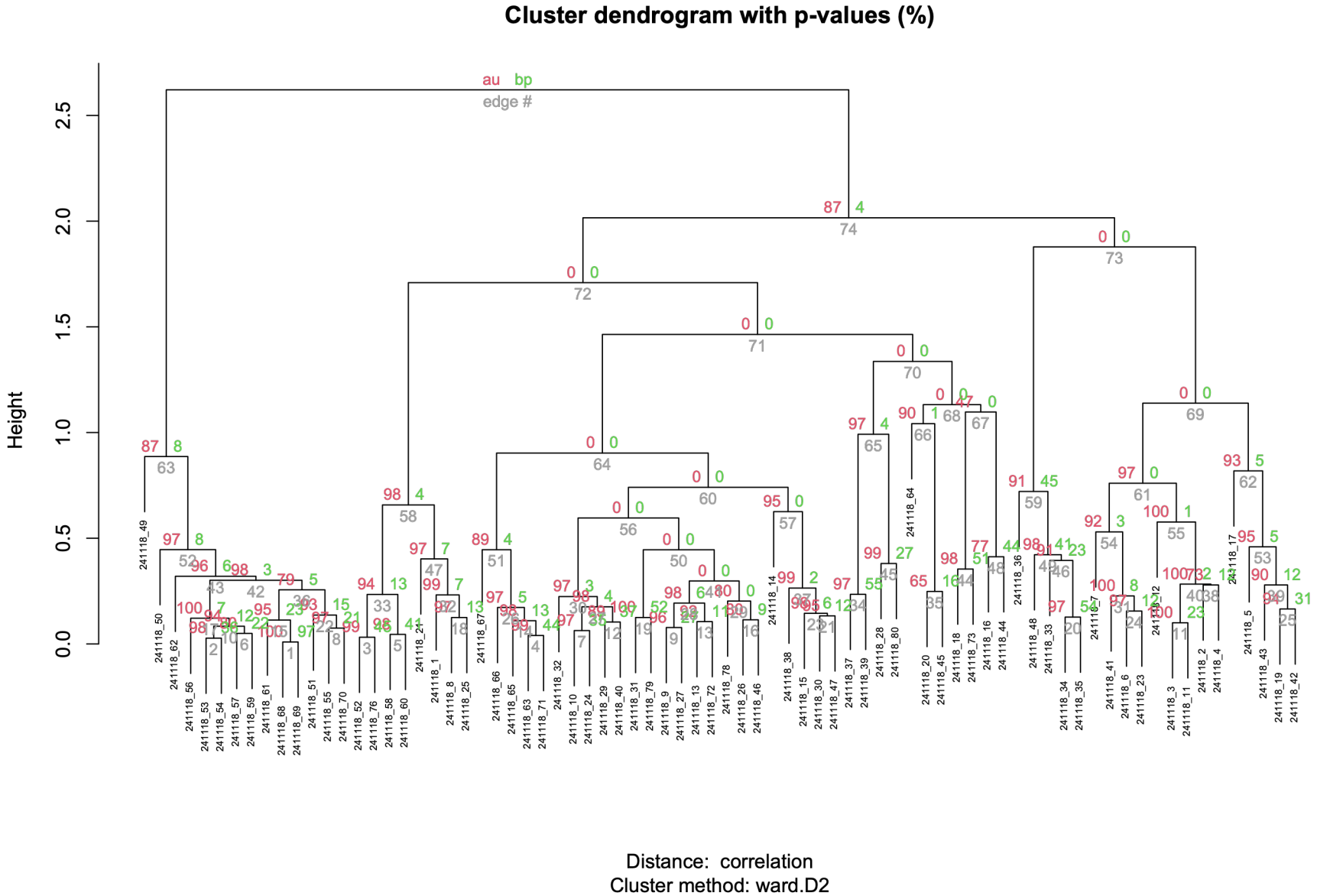

Cluster dendrogram with p-values (%)
au bp
edge #
Height
0.0
0.5
1.0
1.5
2.0
2.5
Distance: correlation
Cluster method: ward.D2

Figure S2

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1260906Fin1.xlsx