

Changes of microbial community during germination of pecan seeds (#88694)

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Changes of microbial community during germination of pecan seeds

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Endophytes are the core of the plant-associated microbiome, and seed endophytes are closely related to plant growth and development. Seed germination is an important part of pecan's life activities, but the composition and changes of microbes ~~in pecan seeds~~ during different germination processes have not yet been revealed. In order to deeply explore the characteristics of endophytes during the germination process of pecan, high-throughput sequencing was performed on seeds at four different germination stages. Through the analysis of diversity and community composition, we found that the diversity and composition of microorganisms were different in different germination stages, and the microbial richness and diversity were the highest in the S3 (seed endocarp break) stage. It was speculated that the change of endophytes in pecan seeds was related to the germination stage. By evaluating the relationship between microbial communities, the core microbiota Cyanobacteria, Proteobacteria and Actinobacteria ~~of bacteria~~ and Anthophyta and Ascomycota ~~of fungal~~ core microbiota were identified. Finally, biomarkers in different germination processes of pecan were identified by LEfSe analysis, among which ~~the bacteria~~ Proteobacteria, Gammaproteobacteria and Cyanobacteria and ~~the fungi~~ Ascomycota and Sordariomycetes were most abundant. This study will help to explore the interaction mechanism between pecan seeds and endophytes in different germination processes, and provide materials for the research and development of pecan seed endophytes.

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ABSTRACT

Endophytes are the core of the plant-associated microbiome, and seed endophytes are closely related to plant growth and development. Seed germination is an important part of pecan's life activities, but the composition and changes of microbes in pecan seeds during different germination processes have not yet been revealed. In order to deeply explore the characteristics of endophytes during the germination process of pecan, high-throughput sequencing was performed on seeds at four different germination stages. Through the analysis of diversity and community composition, we found that the diversity and composition of microorganisms were different in different germination stages, and the microbial richness and diversity were the highest in the S3 (seed endocarp break) stage. It was speculated that the change of endophytes in pecan seeds was related to the germination stage. By evaluating the relationship between microbial communities, the core microbiota Cyanobacteria, Proteobacteria and Actinobacteria of bacteria and Anthophyta and Ascomycota of fungal core microbiota were identified. Finally, biomarkers in different germination processes of pecan were identified by LEfSe analysis, among which the bacteria Proteobacteria, Gammaproteobacteria and Cyanobacteria and the fungi Ascomycota and Sordariomycetes were most abundant. This study will help to explore the interaction mechanism between pecan seeds and endophytes in different germination processes, and provide materials for the research and development of pecan seed endophytes.

Keywords: pecan, seeds, germination process, endophyte microbiota

INTRODUCTION

Seeds are the transmission medium of microorganisms from mother plants to offspring plants, passing beneficial microorganisms from generation to generation. Seed-borne microbes are present in different tissues of the plant and provide a range of benefits to the host plant (Johnston-Monje & Raizada 2013; Lopez-Velasco et al. 2013; Shade et al. 2017). Microorganisms in seeds are diverse, including taxa such as

bacteria, fungi, and archaea (Simonin et al. 2022), and there are about 1-1000 species of bacteria and 1-150 species of fungi in a single seed, and the diversity varies greatly among samples across plant species (Shearin et al. 2018; Truyens et al. 2015). Numerous microorganisms exist in and on seeds, and these microorganisms play important roles in seed storage (Qi et al. 2022a; Qi et al. 2022b), seed germination (Goggin et al. 2015), seedling growth (Gao et al. 2020; Walsh et al. 2021) and plant health (Hu et al. 2020). Endophytes play a direct or indirect role in seed germination to promote the growth of the host plant (Ahmad et al. 2020). Studies have shown that seed endophytes vary by plant species (Links et al. 2014), genotype (Adam et al. 2018), seed maturation process (Mano et al. 2006) and environmental conditions (Klaedtke et al. 2016). Seed endophytes are derived from plant-associated endophytes that can influence plant microbial community structure and function (Midha et al. 2016).

The dominant phyla of bacteria in seeds are Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, the dominant phyla of fungi are Ascomycota and Basidiomycota, and the dominant phyla of archaea are Thaumarchaeota and Euryarchaeota (Simonin et al. 2022; Taffner et al. 2020). Microorganisms widely present in seeds include Pantoea, Pseudomonas, Rhizobium, Dothideomycetes, Tremellomycetes and Candidatus Nitrososphaera and other taxa. Some bacteria can parasitize plant seeds and they may be the creators of rhizosphere or internal bacterial communities early in plant development. These seed endophytes have been shown to have beneficial effects on the host plant, such as releasing seeds from dormancy, promotes seed germination, defends against pathogens and promotes seedling growth (Khalaf & Raizada 2018; Rahman et al. 2018). *Cladosporium cladosporioides* acts as a seed endophyte with properties that promote seed germination and plant growth (Qin et al. 2016). Fan et al. isolated *Arthrobacter* and *Bacillus megaterium* with growth-promoting ability, which can effectively improve the germination rate of tomato seeds, seedling height, fresh weight and dry weight of plants. Moreover, after inoculating rice with good functional strains screened by Fan et al., 4 strains were found to significantly increase the stem length, root length, root weight and plant fresh weight of rice seedlings (Fan et al. 2016). Choi et al. showed that *Capsella bursa-pastoris* seeds harbor bacterial endophytes that stimulate seedling growth, thereby potentially affecting seedling establishment (Choi et al. 2022).

Carya illinoensis is an economic tree species of Juglandaceae (Juglandaceae), also known as American hickory and long hickory. It is native to North America and is one of the world's famous dry fruit tree species (Guo et al. 2020). As an important woody oil tree species, pecan nuts has high benefit and wide application, and has a very high value of promotion and application (Guo et al. 2020). Its fruit is large, the shell is thin, delicious and nutritious, and is deeply loved by consumers all over the world. It is well known that pecan nuts have large seeds, thin shells and many nutrients with less astringency and can be eaten freshly. As a fruit, Pecan, which is also used as a seed, contains rich microbial resources that have not been fully tapped so far. At present, there are not many high-throughput sequencing on the study of endophytes in walnuts, and most of them are limited to the isolation of endophytes. For example, the isolation of endophytes in walnut fruits is used to ferment cotton stalks to hydrolyze sugar liquid to produce oil (Zhang et al. 2014a; Zhang et al. 2014b).

We present the first study identifying the pecan seed microbiome during the germination process. The microbiome niche differentiation of different germination process in pecan is evaluated. We have focused here on two primary issues: (1) Which kinds of bacteria and fungi are responsible for the major processes in pecan seeds? (2) Which bacteria and fungi play a major role in germination and which ones promote

pecan seed germination?

MATERIALS AND METHODS

Plant material, experimental design and sample collection

Fresh pecan seeds, used in the present experiment were provided by the Jiang Tao Family Farm, Lai 'an County, Anhui Province, China in November 2022, and the cultivar is "Pawnee". The seeds were kept at room temperature in Chuzhou University's Plant Physiology Laboratory. For ten days, pecan seeds were imbibed at room temperature. The seeds were then placed in a germination box covered with moist absorbent cotton and placed in a 30 °C constant temperature full light incubator. During seed germination, we sampled four times: S1 (imbibed 10 days), S2 (after 5 days in the incubator), S3 (seed endocarp break) and S4 (seed radicle protrusion). At each stage of germination, we collected 5-10 seeds that germinated consistently and surface sterilized them with 2% NaClO by shaking them for four minutes. In order to eliminate residues of NaClO that may interfere with the next steps, the seeds were rinsed five times in sterile MilliQ water after treatment with NaClO. Finally, all samples were immediately frozen in liquid nitrogen. For microbial sequencing, the samples were stored at -80°C in a refrigerator.

DNA extraction, amplification and sequencing

We extracted total genome DNA from seed samples using the CTAB (cetyl trimethylammonium bromide) technique (Niemi et al. 2001). Analysis of 1% agarose gels for DNA content and purity has been conducted. DNA was diluted to 1ng-μL⁻¹ in sterile water, depending on the concentration. To generate the bacterial libraries, we used the 799F (5'-AACMGGATTAGATACCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') primers set with the unique 6-nt barcode at 5' of the forward primer to amplify the V5-V7 region of the 16S rRNA gene for each sample. The construction of the fungal libraries was similar to the bacterial libraries, except that they were amplified using ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') for the ITS2 region. All PCR reactions were performed using Phusion® High-Fidelity PCR Master Mix (New England Biolabs). One-load buffer (including SYB green) was mixed with PCR products at a 1:1 volume ratio, and electrophoretic detection was performed on 2% agarose gels. The PCR products were mixed in an equal density ratio. The mixed PCR products were then purified using the Qiagen Gel Extraction Kit (Qiagen, Germany). In accordance with the manufacturer's recommendations, sequencing libraries were generated using the Truseq® DNA PCR-Free Sample Preparation Kit (Illumina, USA). Index codes were also added to the libraries. A Qubit® 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system were used to evaluate the quality of the library. An Illumina MiSeq PE300 platform was used to sequence the libraries and generate paired reads.

Sequence analysis

All raw data from the 16S V5-V7 bacterial region and the fungal ITS1 region was processed by QIIME for quality-controlled process (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html), and FLASH for paired reads (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) (Bolyen et al. 2019; Magoč & Salzberg 2011). For bacteria and fungi, annotation was done by matching the Silva sequences with the UCHIME algorithm

and Unite database (ITS: <http://unite.ut.ee/>) (UCHIME, http://www.drive5.com/usearch/manual/uchime_algo.html) (Koljalg et al. 2013; Quast et al. 2012).

Statistical analysis

For the analysis of Alpha Diversity, four indices of species diversity are applied, including Observed Species, Chao1, Shannon, and Simpson. These indices were calculated using QIIME (Version 1.9.1) and visualized using R package ggplot2 (Version 2.15.3). One-way ANOVA was used to determine significant differences at a *P*-value of 0.05; if significant differences were detected, Duncan's post hoc test was used to identify the values that differed from the others. A Beta diversity analysis was used to determine the differences in species complexity among samples, and Beta diversity on Bray-Curtis was calculated using QIIME software (version 1.9.1).

RESULTS

Alpha and beta-diversity of pecan seed microbial communities

Pecan seeds were sequenced using the Illumina MiSeq high-throughput platform to determine the diversity of bacterial and fungal communities and their ASVs. The Good's coverage index of all samples was greater than 98.5%, indicating that the sequencing results could accurately reflect the true status of the bacterial and fungal communities within the samples. In terms of Shannon diversity index, bacteria had a higher diversity of S3 (seed endocarp break), whereas fungi had a higher diversity of S1 (imbibed 10 days). Both the ACE index and the Chao1 index of seed endophyte microorganisms in the four stages have the same trend, and S3 is the highest, indicating the most abundant species in the S3 stage. Additionally, the species richness of the S4 (seed radicle protrusion) stage in fungi was significantly lower than that of the other samples. It was found that the Pielou evenness index of bacteria was generally small, indicating that the species distribution of bacteria in pecan seeds was not uniform. Generally, the fungal index is close to 1, indicating that fungal species are equally distributed, and the S1 stage species distribution is the most uniform (Table 1).

PCA is a two-dimension reduction ordination analysis method based on Bray–Curtis distance for assessing the similarities and differences between bacterial and fungal communities (Figure 1). We performed PCA in our study in order to investigate the separation of bacterial (Figure 1a) and fungal (Figure 1b) communities. In bacterial PCA, 36.49 % of PC1 and 20 % of the total variance were explained by PC2. In fungal PCA, PC1 accounted for 36.01 % and PC2 for 23.24 % of the overall variance.

Microbial community composition in pecan seed germination process

According to the analysis of ASV annotation results, different germination stages of pecan seeds bacteria include 12 phyla, 18 classes, 38 orders, 60 families, 82 genera and 62 species (Figure 2). Among all sequences, the dominant bacterial phyla (relative abundance > 1%) were Cyanobacteria, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Acidobacteria, Euryarchaeota, Deniocooccus-Thermus, Spirochaetes, Ignavibacteriae, Parcubacteria. It can be seen from Figure 2a-e that the endophytic bacteria in the seeds under the S3 and S4 stages are very different from those in the previous two stages. At the phylum level, the two main phyla have undergone major changes, and Cyanobacteria in the first two

stages. The relative abundance of the first stage is relatively large, and in the latter two stages, the relative abundance of Proteobacteria increases, while the abundance of Cyanobacteria decreases. The S3 and S4 stages represent two important stages of seed germination, respectively, and they require different metabolites at the bacterial community level. So, we found that in these two stages, their bacterial communities at different levels have great differences and changes.

Changes in the fungal relative abundance of the major families during germination are shown in Figure 3. Pecan seeds fungi include 7 phyla, 14 classes, 23 orders, 30 families, 35 genera and 22 species. Among all sequences, the dominant fungal phyla (relative abundance > 1%) were Anthophyta, Ascomycota, Basidiomycota, Cercozoa, Bacteroidetes, Chlorophyta, Chytridiomycota, and Mortierellomycota. Among other species, we observed Juglandaceae, an endophyte closely related to pecan.

Core and specific microbiome of pecan seeds

In order to assess the relationship between bacterial and fungal communities, we represented the ASVs shared by different germination stages of pecan seeds as an UpSet plot in Figure 4a-b. UpSet plots are used for visualizing the number and overlap of different ASVs. The results showed that the majority of bacterial ASVs were shared by all the samples (172 ASVs, Figure 4a). 40 ASVs were shared by S1, S3, and S4; 17 ASVs were shared by S1-S3; 24 ASVs were shared by S2-S4. Compared with other stages, S3 stage had the most unique ASVs. From the 180 of the total fungal observed ASVs in all four germination stages, 64 ASVs (35.6% of the total ASVs) were shared between them (Figure 4b). S3 stage shared the highest number of fungal ASVs (37 OTUs, which is 20.6% of the total ASVs). ASVs that were unique to each community represented 4 ASVs for S1 (2.2% of total ASVs) and 3 ASVs for S4 (1.7%). The Venn network diagram used to evaluate the distribution of ASVs among different stages. The core microbiome of the pecan seeds was further analyzed on the phylum level. The common core bacterial microbiome of pecan seed consisted of members of Cyanobacteria, Proteobacteria, and Actinobacteria (Figure 4c). But in fungal, the common core microbiome is Anthophyta and Ascomycota.

To further explore the effects of different germination stages of pecan seed on microbial communities, we analyzed bacterial and fungal communities using samples subjected to S1, S3 and S4 treatments, which affected bacterial and fungal diversity most strongly. The ternary plot showed that high-abundance ASVs belonging to Proteobacteria and Actinobacteria phyla were detected upon S3 and S4 treatments, but the relative abundances of these ASVs in the S1 were extremely low. Moreover, the relative abundances of most ASVs in S3 were lower than those in the S4 (Figure 5a). Next, we used samples from the S1, S3 and S4 treatments to further study the fungal community changes. As observed in the fungal community, a high abundance of ASVs from Ascomycota phylum was observed in the S3 and S4 treatments, and the relative abundance of most ASVs in the S1 group was lower than that in the S3 and S4 treatments (Figure 5b).

Changes biomarkers in the relative abundances of different germination stages in pecan seeds

In our study, linear discriminant analysis (LDA) effect size (LEfSe) analysis was conducted to identify bacterial and fungal as biomarkers (Figure 6). Compared to the seed bacterial community (2 classes, 5

orders, 6 families, 9 genera and 9 species), we found significantly richer biomarker species in the fungal communities (5 classes, 4 orders, 9 families, 10 genera and 12 species) of pecan seeds in different germination stages. Among all taxonomic levels of seed bacteria, 10 were associated with S2, 29 were associated with S3, 14 associated with S4. Among them, Proteobacteria, Gammaproteobacteria and Cyanobacteria were identified as highly abundant biomarkers in the bacterial community. Among all taxonomic levels of seed fungi, 28 biomarkers were related to S1 and 18 were related to S2, 43 biomarkers were related to S3, 12 related to S4. Ascomycota and Sordariomycetes were significantly abundant in the seed fungal community.

DISCUSSION

The germination ~~stage~~ of pecan seeds affected the diversity and abundance of internal microorganisms. In this study, through the analysis of the microbial community diversity and community composition ~~in~~ four different germination stages of pecan seeds, ~~it was~~ found that the microbial richness and diversity were the highest in the S3 stage. The diversity and abundance of bacteria and the abundance of fungi gradually increased from the water absorption stage to the endocarp rupture, and decreased when the radicle protruded, and we speculated that this trend shift was related to the endocarp dehiscence. We speculated that this change of trend was related to the dehiscence of the endocarp. Previous studies have shown that the increase in the relative abundance of symbiotic microorganisms during seed germination and emergence is due to the spermosphere formed during seed germination providing efficient nutrients for the growth of microbial groups in seeds, which originally existed in dormant forms inside the seeds.

Microbes, which break dormancy with nutrients released by germinating seeds (Barret et al. 2015; Kwan et al. 2015; Roberts et al. 2000; Torres-Cortés et al. 2018).

Endophytic bacterial biomarkers Proteobacteria, Gammaproteobacteria and Cyanobacteria were identified at different germination stages of pecan seeds by LEfSe analysis, and we speculate that they play an important role in the seed germination process. Among them, Cyanobacteria and Proteobacteria have high abundance, which ~~is~~ similar to many research results (Chen et al. 2018; Liu et al. 2013; Zhang et al. 2012). During seed germination, Cyanobacteria and Proteobacteria constituted almost the entire bacterial community and were also identified as core and specific microbiomes. Although the relative abundance of Cyanobacteria decreased during S3 and S4, it always maintained the highest relative abundance. The relative abundance of Proteobacteria increased rapidly in the S3 stage and remained in the S4 stage.

Cyanobacteria has been proven to be a beneficial microorganism that can improve plant nutrient uptake capacity, promote growth, and enhance plant tolerance to stress (Rady et al. 2018). In addition, the promotion effect of Cyanobacteria on seed germination and growth has also been affirmed. For example, ~~Seifikalhor et al. used~~ Cyanobacteria to treat maize seeds and found that it has a good effect on promoting maize growth, photosynthesis and anti-Cd toxicity (Seifikalhor et al. 2020). Abdel-Hafeez et al. found that sunflower seeds treated with Cyanobacteria showed higher yield and better growth status (Abdel-Hafeez et al. 2019). However, the effects of bacteria Proteobacteria and Gammaproteobacteria ~~from~~ Proteobacteria on seed germination have been rarely reported, and further research is needed.

Endophytic fungal biomarkers at different germination stages of Pecan seeds ~~were Ascomycota and Sordariomycetes~~. Sordariomycetes is a class belonging to the phylum Ascomycota, and its relative abundance increases gradually after the seeds absorb water until the endocarp of pecan seeds ruptures.

Therefore, we speculate that Sordariomycetes and Ascomycota are ~~endophytic fungi that play an~~ important role in pecan seed germination. Sordariomycetes is the main dominant class of endophytic fungal communities in plants with antipathogenic activity (Ettinger & Eisen 2020; Wang et al. 2019; Zhang et al. 2021). Ascomycota is the most abundant phylum annotated among endophytic fungi at each stage of pecan seed germination, and it is also one of the core and specific microbiomes of endophytic fungi identified in this study. However, no studies have thoroughly elucidated the effects of the seed endophytic fungi Ascomycota and Sordariomycetes on germination.

Seed germination is a complex process in which initially dormant seeds undergo a series of active changes in physiological state (Huang et al. 2018). The abundant microorganisms inside the seeds improve the access to nutrients and enhance the ability of seeds to resist pathogens and abiotic stresses by interacting with seeds (Dai et al. 2020; Morella et al. 2019; Seifikalhor et al. 2020). These positive effects on various physiological activities of the host allow the host to adapt to changing environmental conditions, especially in early life stages (War et al. 2023). Seed endophytic microbes can promote seed germination and seedling growth, and the transition from seed to shoot is marked by changes in bacterial and fungal community composition and increased dominance (Verma et al. 2019; Walitang et al. 2017). Therefore, it is of great significance to explore the relationship between seed germination stages and endophytic microorganisms. In this study, high-throughput sequencing of pecan seeds at different germination stages was carried out, and the diversity and abundance changes of endophytic microorganisms in different germination stages of pecan seeds were studied by combining various analysis methods, and the core microorganisms and microbial markers in the seed germination process were found. It will help to explore the interaction mechanism between seeds and microorganisms at different germination stages in the future, and lay a foundation for the development and research of endophytes in pecan seeds in the future.

CONCLUSIONS

~~We conclude that the~~ diversity and composition of microorganisms were different in different germination stages, and the microbial richness and diversity were the highest in the S3 (seed endocarp break) stage. It was speculated that the change of endophytes in pecan seeds was related to the germination stage. Besides, changes of endophytic microorganisms in different germination stages of pecan seeds were studied, and the core microorganisms and microbial markers in the seed germination process were found. This study will help to explore the interaction mechanism between pecan seeds and endophytes in different germination processes, and provide materials for the research and development of pecan seed endophytes.

ACKNOWLEDGMENTS

We are grateful to all study participants for their contributions. We also thank the Language Editing Service of Keteng Edit for editing our manuscript.

ADDITIONAL INFORMATION AND DECLARATIONS

Ethics approval and consent to participate

Our plant materials were collected from the Jiang Tao Family Farm. These plants are not protected; therefore, permission is not required for sample collection. In addition, the place of sample collection is not a protected area, therefore no any legal authorization/license is required. All authors have participated the work and comply with the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Consent to publication

Not applicable.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Jia Liu: Conceptualization; Data curation; Investigation; Resources; Software; Visualization; Writing-original draft; Writing-review & editing. Sumei Qiu & Liping Yang: Investigation; Software; Visualization; Funding acquisition; Writing-review & editing. Tingting Xue& Yingdan Yuan: Conceptualization; Resources; Supervision; Validation; Writing-review & editing. All the authors have read and approved the study.

Funding

This research work was funded by the National Natural Science Foundation of China (Grant No. 32001310), Anhui Provincial Educational Foundation (2022AH030111) and Chuzhou University Start-up Foundation for Research (2020qd33, 2021qd05).

Data availability

Raw Illumina sequence data was stored for bacterial 16S and ITS fungal data in the archives of the NCBI Sequence Read with accession numbers PRJNA940091 (Bacterial) and PRJNA940387 (Fungal).

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Figure 1

Beta-diversity of different germination stages in pecan seeds

Principal analysis (PCA) of bacterial (a) and fungal (b) communities among different germination stages

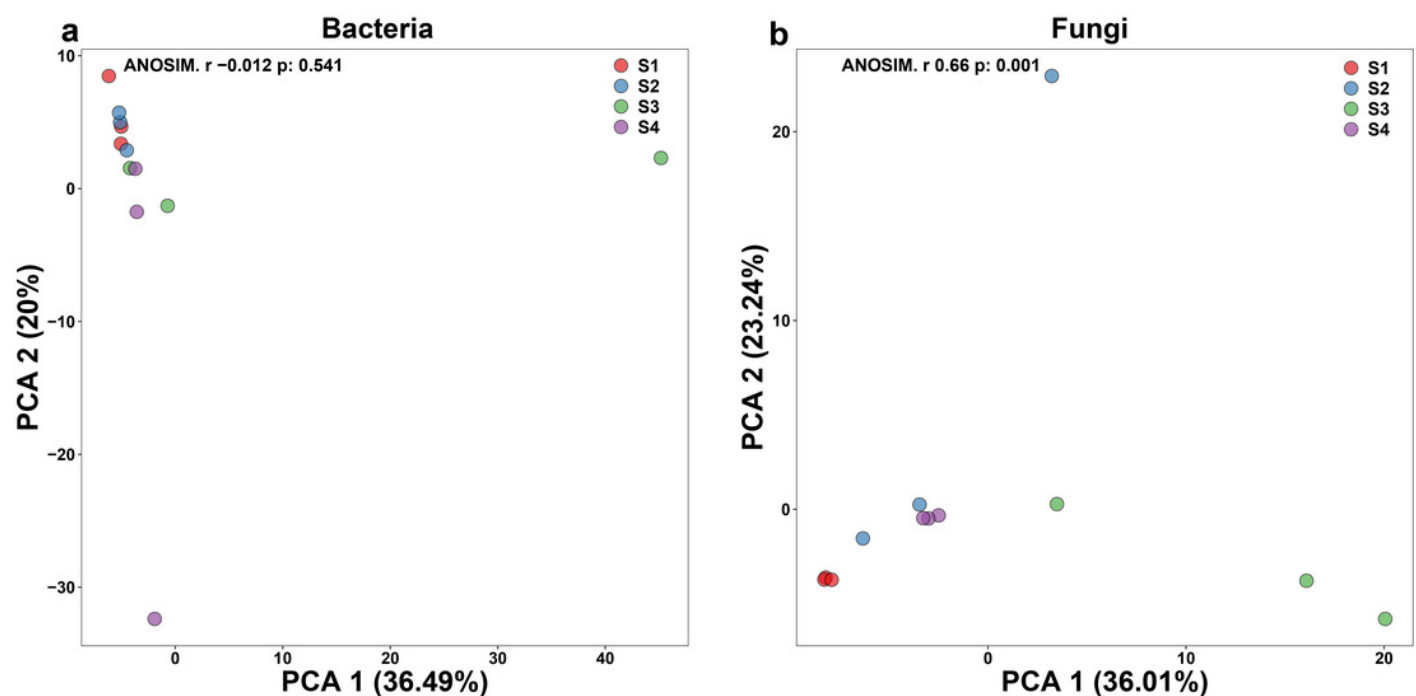


Figure 2

Top 10 relative abundances of bacterial communities classified at phylum (a), class (b), order (c), family (d) and genus (e) level in different germination stages of pecan seeds.

phylum (a), class (b), order (c), family (d) and genus (e)

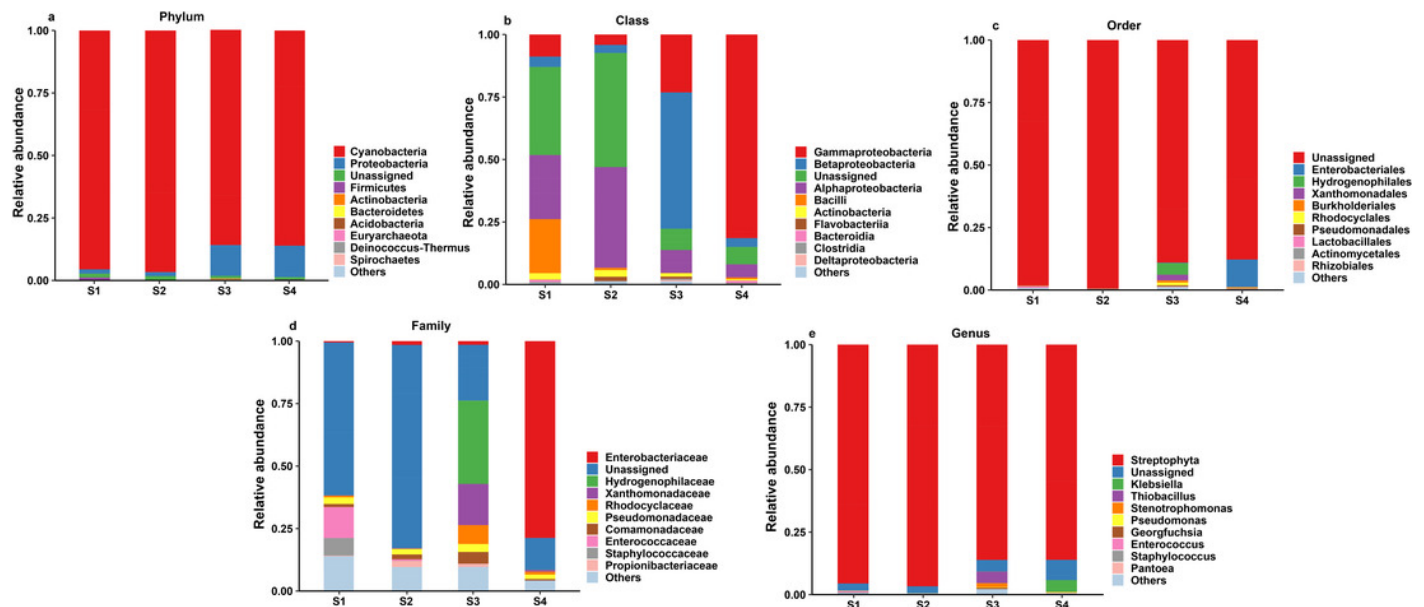


Figure 3

Top 10 relative abundances of fungal communities classified at phylum (a), class (b), order (c), family (d) and genus (e) level in different germination stages of pecan seeds

phylum (a), class (b), order (c), family (d) and genus (e)

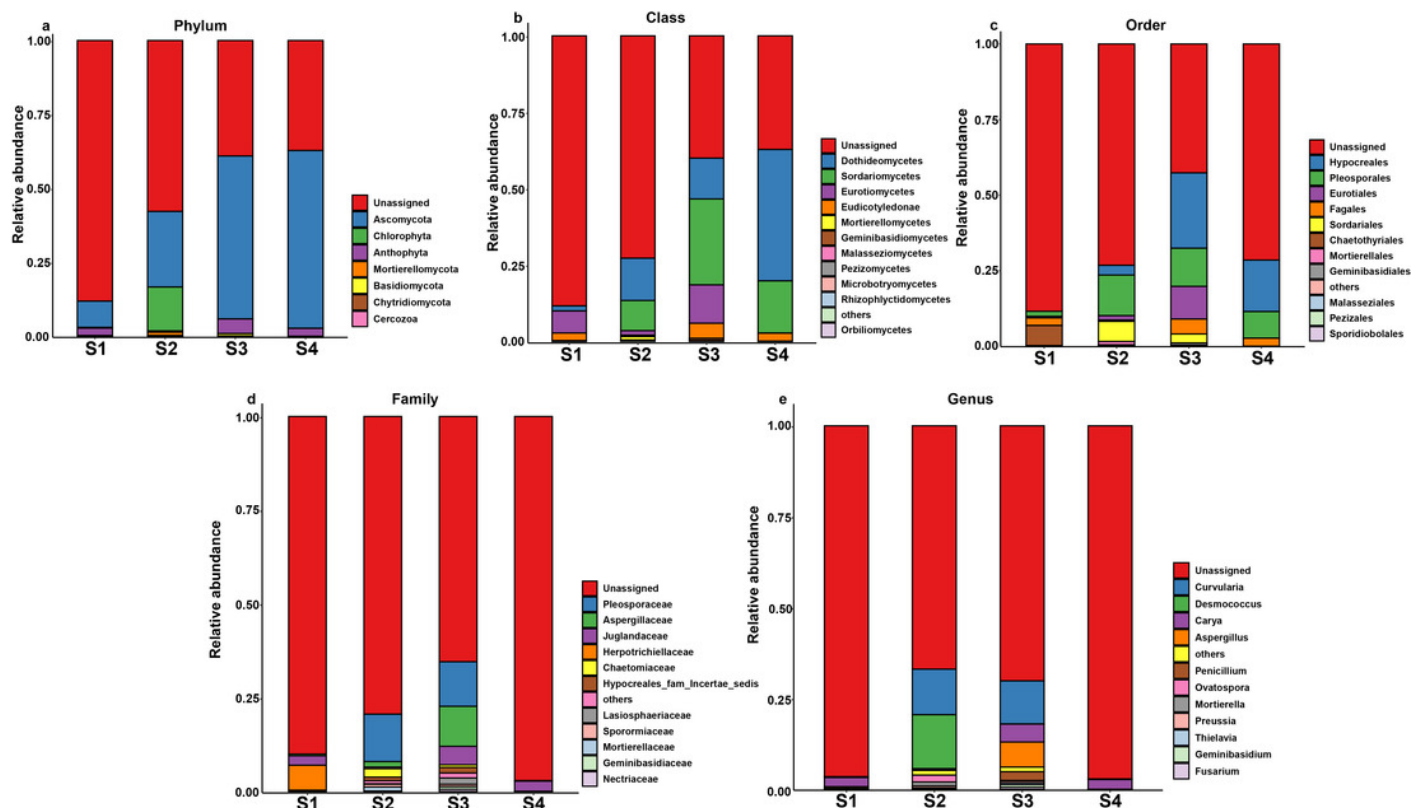


Figure 4

UpSet and Venn network diagram

(a-b) Upset diagram representing bacterial (a) and fungal (b) ASVs associated with the pecan seed microbiome; (c-d) Venn network diagram, with nodes (ASVs) colored according to each of the main ecological clusters: (c) Bacterial, (d) Fungal

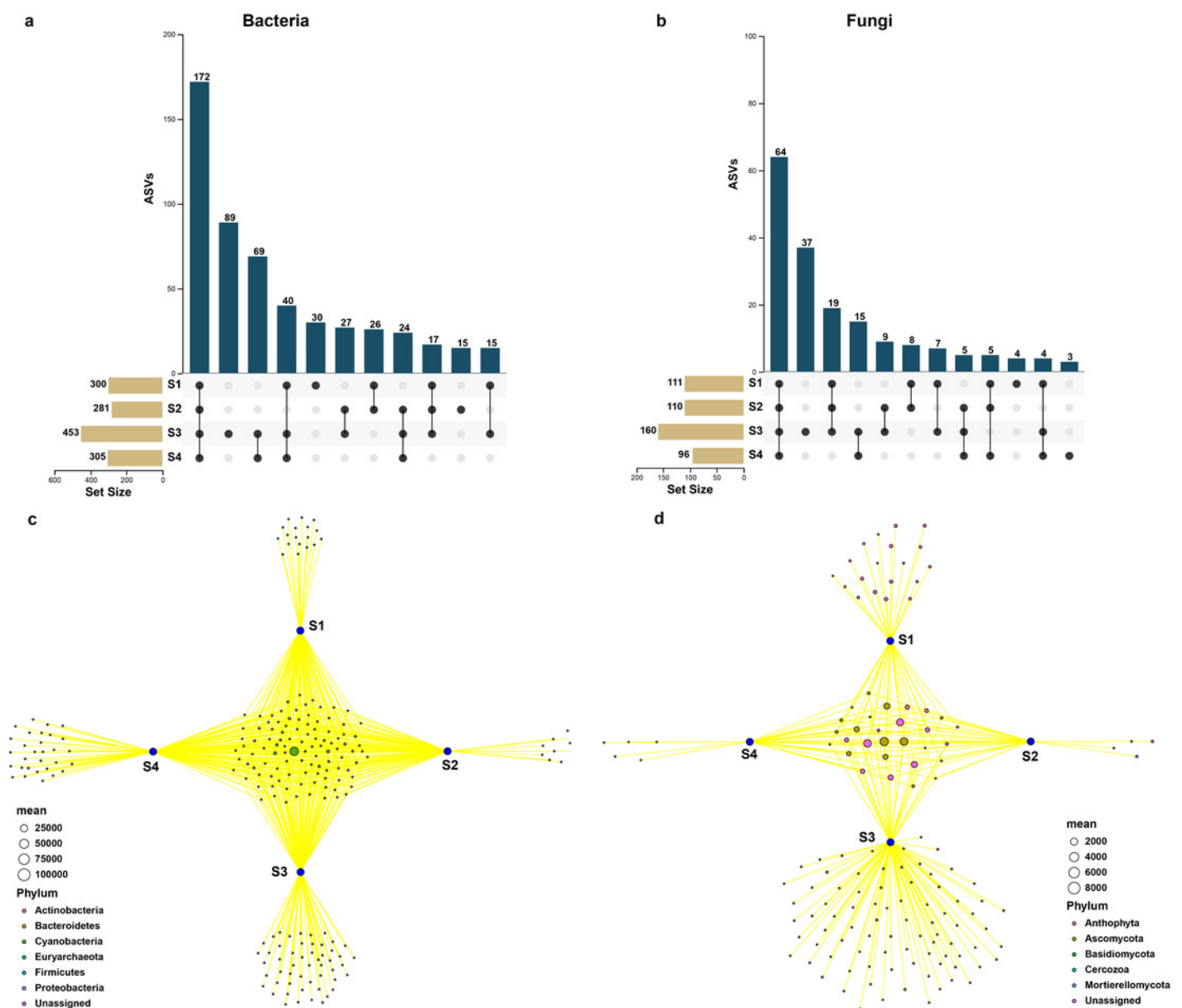


Figure 5

Ternary plot of bacterial ASV distribution for S1, S3, and S4 stages (a), and fungal ASV distribution for S1, S3, and S4 stages (b)

Each point corresponds to an ASV. The position represents the relative abundance with respect to each treatment, the size represents the average across all three treatments, and the color represents bacterial or fungal phylum

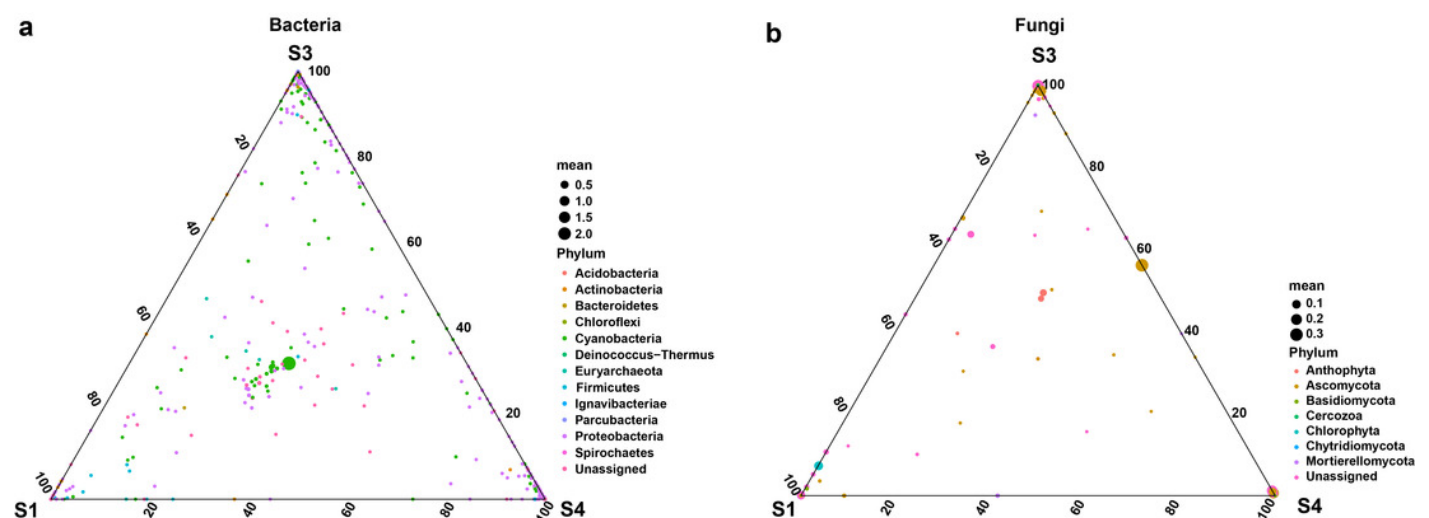


Figure 6

Linear discriminant analysis effect size (LEfSe) of the bacterial (a) and fungal (b) communities with an LDA score higher than 4.0 and P-values <0.05

bacterial (a), fungal (b)

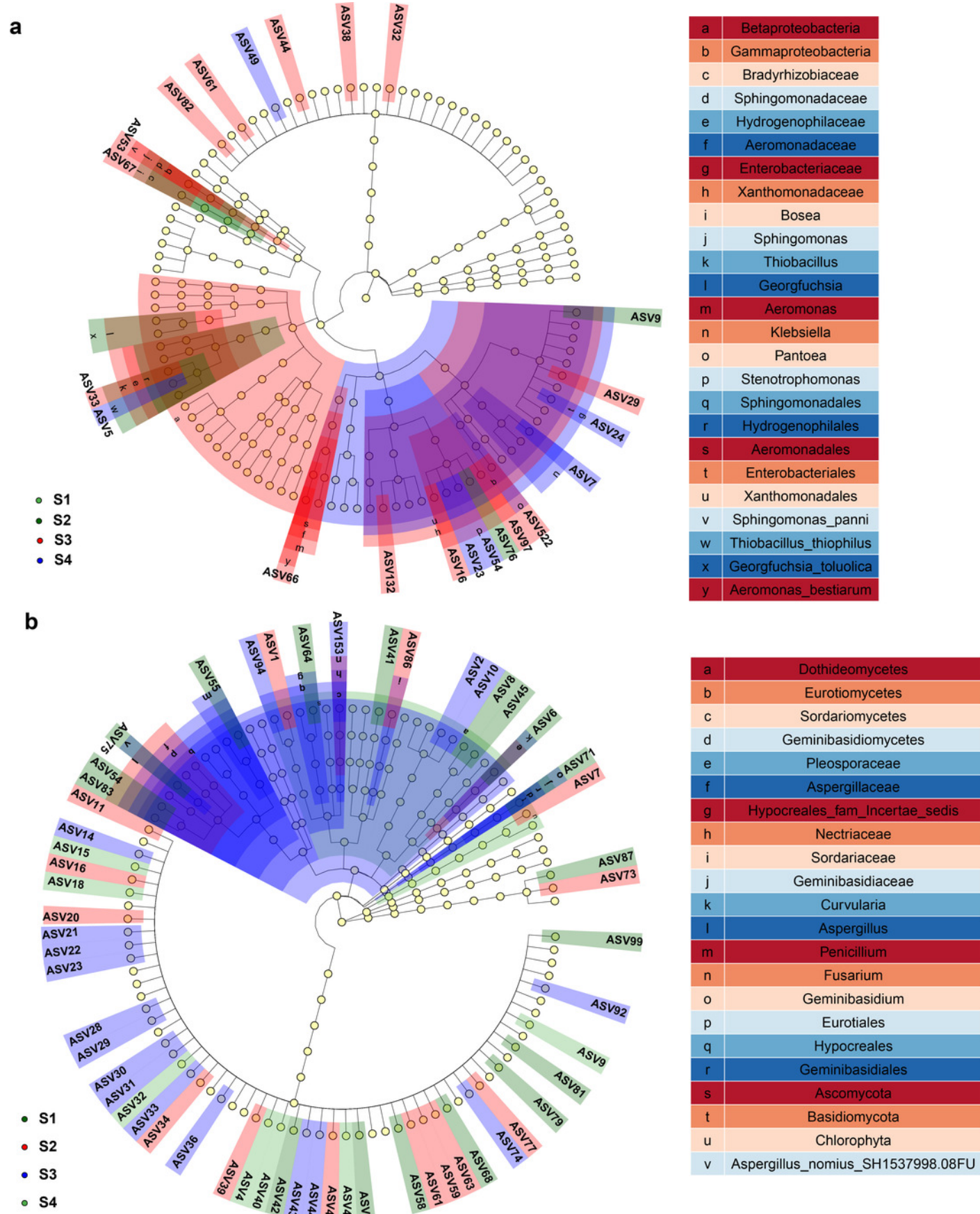


Table 1 (on next page)

Alpha-diversity estimates of samples

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1

Table 1 Alpha-diversity estimates of samples

Microbial	Sample ID	Shannon	Pielou evenness	Chao1	ACE
Bacteria	S1	0.8 ± 0.15^a	0.16 ± 0.03^a	192.92 ± 20.15^b	193.59 ± 17.15^b
	S2	0.77 ± 0.15^a	0.15 ± 0.03^a	197.94 ± 5.82^{ab}	190.23 ± 3.55^b
	S3	1.38 ± 1.09^a	0.24 ± 0.18^a	284.55 ± 80.58^a	283.08 ± 80.87^a
	S4	1.18 ± 0.75^a	0.22 ± 0.13^a	235.55 ± 35.95^{ab}	236.39 ± 37.26^{ab}
Fungi	S1	3.54 ± 0.05^a	0.82 ± 0.03^a	83.34 ± 13.81^b	82.4 ± 11.26^b
	S2	2.66 ± 0.38^b	0.59 ± 0.09^b	103.47 ± 25.93^{ab}	105.83 ± 23.57^{ab}
	S3	2.67 ± 0.21^b	0.54 ± 0.05^b	149.59 ± 44.38^a	149.99 ± 42.97^a
	S4	1.43 ± 0.42^c	0.38 ± 0.1^c	55.91 ± 13.31^b	58.66 ± 11.88^b

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