

Microbial's Effects on Invasive Grass' Response to Water and Nutrient Stress

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Abstract

Background Soil microbiomes play important roles in invasion biology, yet it is often treated as a 'black box' in modeling or large-scale field studies. Hence, investigating the change of association between invasive vegetation and soil microbes under changing environmental conditions, and exploring the genetic functions of associated microbiomes will provide a deeper understanding of invasion mechanisms. We performed a microcosm experiment with cogongrass (*Imperata cylindrica* (L.) P. Beauv.), which is one of the 100 worst invasive plants in the world. We combined rigorous sequencing analysis, including 16S rRNA, ITS, and shotgun metagenome sequencing, for the first time, to investigate the interactive effect of change in soil water and nutrient concentrations on microbiomes diversity, composition and genetic functions under invasion.

Results We found that experimental drought has a stronger effect on the bacterial community than the fungal community. We discovered an enrichment of microbial groups, including Proteobacteria, Actinobacteria, Bacteroidetes and Chloroflexi under drought treatment, could likely contribute to invasion success. Further, we showed a striking trend of induction of cell wall, membrane and desiccation-related genes in drought treatment and a marked downregulation in regular treatment, which could create a more hydrated microenvironment, facilitating biofilm formation and better protection from desiccation.

Conclusions Our work contributes to highlighting the associated microbial communities may have a potential long-term impact on increasing cogongrass drought resistance, ultimately, future invasion might be severe due to the plant-microbe interaction. These findings are important because current modeling practice, lacking comprehensive consideration of the plant-microbe interaction, could lead to a significant underestimate of predictions of future invasion patterns.

Background

Ecosystems are facing substantial threats from biological invasions that are further promoted by the increased stresses of the Anthropocene (e.g., climate change) (1, 2). For example, rangelands throughout the world are encountering non-native grass invasion, especially C_4 grass, which have greater drought tolerance, and high-temperature adaptations, compared to native grasses (3). Similar to drought, which is likely to be more frequent, severe, and longer-lasting (4), global nitrogen deposition is another increasing environmental problem, which alters soil nitrogen concentration and biogeochemical cycling (5, 6). Previous studies have investigated how invasive plants respond to a gradient of soil water and nutrients, respectively (7-9), whereas the interactive effect of change in soil water and nutrients is still poorly understood. Hence, this study aims to fill in an important gap in understanding the interactions among invasions, drought, and nutrient addition – the three most fundamental global drivers of change and their microbial-to-vegetational responses and underlying mechanisms.

Soil microbiomes are known to play important roles in invasion biology (10). Hence, assessing the change of soil microbes under a gradient of soil water and nutrient will provide a complete understanding

of invasive plant response to environmental stress. Soil water and nutrient availability are known to affect the root-associated microbial communities (11). For instance, drought may affect the root microbiome directly by lowering moisture availability, as well as indirectly by altering the chemical properties of the soil, and affecting the physiology of the plant host (12). Correspondingly, soil microbes are important contributors to ecosystem services as they are intrinsically linked with plants and nutrient cycling through their roles as decomposers, mutualists, pathogens, and so on (13, 14). For example, plant growth-promoting rhizobacteria (PGPR), in particular, represent a wide range of root-colonizing bacteria with excellent root colonizing ability and capacity to produce a wide range of enzymes and metabolites that help plants tolerate both biotic and abiotic stresses (15). However, in most cases, soil microbiomes are treated as a 'black box' in many modeling (16), or large-scale field observation studies (10), especially in terms of the functional roles of different members of the microbial community (17).

Investigating the functional roles of soil microbes may provide potential explanations for the success of plant invasions, especially under shifting environmental conditions (18, 19). Despite research focus has shifted to below-ground mechanisms of invasion, which has provided valuable insights into the role of soil microbes in invasion (20), the field is still in the early stage. One possible mechanism is the alterations in soil microbial communities, which can be exacerbated by climate change (21, 22). For instance, shifts in microbial community composition and bacterial diversity under drought could be linked to changes in biogeochemical processes that influence the availability of resources (23), whereas current findings are inconsistent (24, 25). An experiment in semi-arid grasslands showed that soil microbial community structure is unaltered by plant invasion and nitrogen fertilization (26). Conversely, another study showed that plant communities mediate the interactive effects of invasion and drought on soil microbial communities (21).

More importantly, previous studies have mainly examined the pattern of microbial abundance and composition during invasion, the underlying mechanism and microbial functions are less explored. Investigating the change of association between invasive vegetation and soil microbes, and exploring the genetic functions of associated soil microbes, will provide a deeper understanding of invasion mechanism, ultimately, could suggest a better field management strategy. The most modern methods for studying shifts in soil microbiota communities include shotgun metagenome sequencing analysis where chronometer genes, not subject to horizontal gene exchange in bacteria and fungi, offer a great opportunity to analyze the microbiome and their functional dynamics (27, 28).

To identify the interactive effect of change in soil water and nutrient concentrations on soil microbial communities under biological invasion, we conducted a microcosm study with the model species of cogongrass (*Imperata cylindrica* (L.) P. Beauv.). Cogongrass is a severe invasive C₄ grass that has been listed as one of the 100 worst invasive plants in the world and listed as Federal Noxious Weed in the U.S. (29). Specifically, we conducted factorial experiments that manipulated soil water and nutrient contents of cogongrass' growing environment and demonstrated microbial shifts in community composition and functional capacities via using 16S rRNA gene (bacteria) and ITS (fungal) sequencing, and metagenome sequencing analysis. This study aimed to address the following four questions: (1) How do changes in

soil water and nutrient content impact soil microbial diversity and abundance? (2) What are the links between microbial communities and plant performance (as an indication of invasion success)? (3) How does soil microbial community structure change in response to changes in the environment? (4) What are the important microbial gene families and their functions responding to water and nutrient stress?

Results

1. The effects of treatments on soil microbial diversity and abundance

The ranges of high-quality sequences in rhizosphere soil obtained were 70,494 to 80,251 reads/sample for bacteria (16S) belonging to 4429 distinct OTUs (Fig. 1a) and 80,004 to 80,239 reads/sample for fungi (ITS) belonging to 1473 OTUs (Fig. 1d). Analysis of the α -diversity index of fungi and bacteria in cogongrass rhizosphere soil showed that the good's coverage of all treated bacteria and fungi samples was greater than 99%, indicating that most of the sequences in the samples were detected and the data obtained realistically describe the composition of these microbiomes (Fig. S4, a and e). Overall, bacterial diversity was greater than fungal diversity across all the treatments and all diversity indices and effects of our treatments on α -diversity differed substantially between bacterial and fungal communities (Fig. 1, b, c, e and f, S4, b-d and f-h). Under high nutrient conditions, bacterial diversity significantly decreased in the low water treatment (LW,HN) (Simpson index: 0.0234; Fig. 1c), whereas under low nutrient conditions, low water treatment significantly increased diversity of fungi (LW,LN) (Simpson: 0.001; Shannon: 0.0061). Similarly, the low water and high nutrient treatment (LW,HN) had significantly higher fungal diversity than the high water and low nutrient treatment (HW,LN) (Simpson: 0.0211). Lastly, under high water conditions, we also detected significant higher fungal diversity in the higher nutrient treatment (HW,HN) (Simpson: 0.0015; Shannon: 0.006; Fig. 1e and f).

2. Links between microbial communities and plant performance

The SEM model adequately fit the data describing interaction pathways among plant, soil, and microbial variables (standardized path coefficients are given in Fig. 2). Despite the negative relationship between soil water content and microbial taxonomic composition (standard coefficient = - 0.168, $P < 0.01$) (Fig. 2), we found a significant positive relationship between soil moisture and microbial gene richness (standard coefficient = 0.175, $P < 0.01$) (Fig. 2), suggesting that while higher soil moisture reduced diversity at the species level it increased diversity at the genetic level. Additionally, below-ground biomass was negatively associated with microbial gene richness (standard coefficient = - 0.677, $P < 0.01$) (Fig. 2), and positively associated with microbial taxonomic composition (standard coefficient = 0.671, $P < 0.01$) (Fig. 2). Together, the significant lower belowground biomass production under low water treatment (5.75 0.47 g in low water treatments, and 10.52 1.15 g in high water treatments, nutrient treatment and the interaction of water and nutrient did not show significant effects on the belowground biomass) could be explained by the increase of microbial gene richness and the deduction of microbial taxonomic composition resulted from the low water treatment.

3. The change in soil microbial community structure in response to changes in environment

According to the analysis of OTU annotation results, cogongrass rhizosphere soil bacteria included 2 kingdoms, 40 phyla, 49 classes, 103 orders, 194 families, 362 genera and 202 species.

The fungal composition included 1 kingdom, 10 phyla, 34 classes, 81 orders, 154 families, 219 genera and 203 species. Due to the second-generation sequencing, OTU can only be targeted to genera, the number of species levels will be relatively small (57). The composition and relative abundance of top 10 soil bacteria and fungi at different levels are shown in Fig. 3 and S5. The composition of the total bacterial community was dominated by the following phyla: Proteobacteria (46.11 %), Actinobacteria (26.2 %), Bacteroidetes (5 %), Acidobacteria (6.24 %), Verrucomicrobia (2.59 %), Chloroflexi (2.96 %) and Planctomycetes (3.4 %). Dominant fungal taxa included: Basidiomycota (56.9 %), Ascomycota (29.36 %), Glomeromycota (0.36 %), Mortierellomycota (0.3 %), and Rozellomycota (0.25 %) (Fig. 3).

We identified 148 taxa that distinguish the four environmental treatments with linear discriminant analysis scores of greater than 4 (Fig. 4 and S6). Among the four treatments, low water and high nutrient (LW,HN) treatment had the most significant different species, which include 93 species. Interestingly, although the fungi were more responsive to our treatments than bacteria in our diversity analysis, in this analysis we found that for all treatments the biomarkers were mainly bacteria.

4. Important gene families and their functions responding to water and nutrient stress

Based on the functional annotation of metagenomes sequencing analysis, we discovered an overarching trend of taxannotation patterns between low-water treatments (LW,LN and LW,HN) and high-water treatments (HW,LN and HW,HN) (Fig. 5a), which can be contributing to cogongrass remarkable invasion success. In the low water treatments, Proteobacteria and Actinobacteria were the two most abundant phyla, including *Rhizobiales*, *Devosia* sp., *Pseudolabrys* spp., and *Hyphomicrobium denitrificans* representing Proteobacteria, and *Acidothermus cellulolyticus*, *Solirubrobacterales*, etc representing Actinobacteria. We noted an increased abundance of the only Chloroflexi taxa detected in our experiment, *Ktedonobacter racemifer*, in the low water low nutrient samples, while Verrucomicrobia species, such as *Opitutus* sp., *Lacunisphaera limnophila* and *Pedosphaera parvula*, were depleted in soil samples obtained from low water conditions. Additionally, we observed a mixed trend for Proteobacteria, with specific bacterial genera increasing or decreasing in response to drought and others changing their abundance in response to relative nutrient availability.

Furthermore, we observed that Acidobacteria's abundance decreased under low water conditions, irrespective of the nutrient status (LW,LN and LW,HN samples). While we found that bacteria from the order Sphingobacterales (Bacteroidetes) generally responded positively to HW,HN conditions. For instance, *P. terrae* thrived under LW,LN treatment, which could be attributed to its unique metabolic ability to tolerate salinity as described above, and make it a flagship example of an ultimate microbial strategist in the recently proposed Y-A-S theory (high yield – resource acquisition – stress tolerance) (58).

Based on the Carbohydrate-Active enZymes database, we gathered insights into the main carbon-centered metabolic activities of our microbiome samples as a function of water and nutrient accessibility. We noted a strong enrichment in genes related to cell wall structure, including cell rigidity and surface, membrane stability and desiccation response. The primary responses within the cell wall are aimed at changes in architecture and composition to create a more hydrated microenvironment, facilitating biofilm formation and better protection from desiccation. In addition, the presence of fertilizer resulted in relatively higher levels of genes abundance compared to the low nutrient samples, indicating that the soil used for our experiment was likely nutrient-limited, or that the increased availability of nutrients promoted host production of root exudates that enhanced bacterial carbohydrate metabolism (Fig. 5B).

Discussion

Our results mapped the interactions among invasions, drought and nutrient addition – the three most fundamental global drivers of change, to underpin the microbial-to-vegetational responses. We discovered an enrichment of microbial groups, including Proteobacteria, Actinobacteria, Bacteroidetes and Chloroflexi under drought treatment, which likely contributes to cogongrass invasion success. Beyond examining the pattern of microbial abundance and composition during invasion, we further investigated microbial genetic functions to understand the underlying mechanism. We showed a striking trend of an induction of cell wall, membrane and desiccation-related genes in experimental drought treatment, and a marked downregulation in regularly watered samples. More importantly, our results suggested that despite the six months experimental drought resulted in significant lower biomass production, the associated microbial communities may have a potential long-term impact on increasing cogongrass drought resistance. Ultimately, our results suggested that future invasion might be severe due to the plant-microbe interaction.

The effects of treatments on soil microbial diversity and abundance

Compared to the change of nutrient condition, experimental drought negatively affected bacterial community diversity more strongly than fungal diversity as we detected a decrease of bacterial Simpson index but an increase of both fungal Shannon and Simpson indices under low water treatment. This result agreed with previous studies with showing that drought decreased alpha diversity, whereas fungal diversity tended to increase (59-61). One possible explanation is that the higher fungal diversity under limited water condition could result from reduced dominance of a particular taxa because carbon flow from plant roots to the soil is reduced during the drought treatment (62), and some normally abundant fungal species might be especially sensitive to that (63, 64). Another explanation is that changes in bacterial communities link more strongly to soil functioning during recovery than do changes in fungal communities (65, 66).

Links between microbial communities and plant performance

Consistent with Zhang, *et al.* (56), who did not find any significant pathways between soil nutrient content (soil NO₃-N) and microbial communities, we showed no significant pathways between soil nutrient content (soil N%) and microbial communities. Furthermore, we detected two significant pathways between soil water content and microbial gene richness and microbial taxonomic composition, and these pathways were not considered in previous studies. Different to Bowen, *et al.* (55) that did not find significant associations between microbial communities and plant productivity, we found below-ground biomass was negatively associated with microbial gene richness, and positively associated with microbial taxonomic composition. Together, these results provide a possible explanation on the significant lower below ground biomass production we found in low water treatments was caused by the impact of experimental drought on microbial communities (67). Additionally, the nonsignificant associations between soil nutrient content and microbial communities, and between soil nutrient content and biomass production further indicated cogongrass is not sensitive to nutrient stress but to water stress. This finding corroborates with previous studies showing that cogongrass can remain competitive under nutrient limitation (68).

The change in soil microbial community structure in response to changes in environment

Based on the metagenome sequencing analysis, we discovered several important microbial phyla that are associated with drought resistance. Actinobacteria and Proteobacteria were the two most abundant phyla with increased presence in our low water soil samples. Both of those have been well documented to produce hopanoids, omnipresent natural products known to contribute to bacterial resilience (69). Most commonly, hopanoids are found in select groups of aerobic bacteria in low-oxygen environments, and the rhizosphere is a niche that is common to many hopanoid-producing bacteria, including Actinobacteria and Proteobacteria - both aerobic and soil dwelling. Hopanoids are thought to play a role in membrane integrity, antibiotic resistance as well as pH and other stress tolerance (70, 71). It is plausible that an increased abundance of a number of bacterial genera found through our analysis in the low water samples can be explained by their ability to increase drought stress resilience through hopanoids biosynthesis. In addition, Actinobacteria have been described to be able to grow under unusually low moisture conditions both in the lab and in natural environment (72, 73), owing to their exceptional ability to adapt transcriptionally as well as the structure and thickness of their Gram-positive peptidoglycan cell wall layer (74).

Furthermore, we found some symbiotic bacteria, such as *Rhizobia* spp. (belonging to Proteobacteria), which is a type of plant growth-promoting bacteria, were strongly enriched in our drought treatment. While cogongrass is not a nitrogen-fixing plant, the presence of these bacteria could stimulate the formation of nodules on the roots of other plants than cogongrass and thus may help reduce environmental stress that plants experience (75, 76). Beyond soil bacterial, we also detected high expression of *Fusarium* spp., and *Alternaria* spp. in low water treatments. These two fungus, which belong to endophytic fungi, reside entirely inside plant tissues and can interact with roots, stems, and leaves, ultimately can improve plant drought tolerance (76).

In a recent study by Dai, *et al.* (77) the abundance of Proteobacteria, Chloroflexi and Verrucomicrobia decreased in drought-treated soil groups compared to control soil of the peanut rhizosphere. Consistently with their observations, we also showed that Verrucomicrobia species were depleted in soil samples obtained from low water conditions. This finding is in agreement with the notion that Verrucomicrobial cells growing on or near the root surface may depend on carbon sources coming from the plant (78, 79), which are expected to be less readily available under drought conditions. In contrast to the findings of Dai, *et al.* (77) but agreed with Naylor, DeGraaf, Purdom and Coleman-Derr (80), we noted an increased abundance of the only Chloroflexi species detected in our experiment, *Ktedonobacter racemifer*, specifically in the low water low nutrient sample. *K. racemifer* is a green non-sulfur bacterium that has the largest genome of all known prokaryotes and the highest number of novel genes (81). This unusually complex genome could offer an evolutionary advantage towards living in water- and nutrient-limited environment for *K. racemifer* itself, but also help make soil nutrients bio-available for the plant host using alternate metabolic pathways.

One of the plant root's primary roles is the absorption of nutrients, thus it is expected that the microbial communities associated with the rhizosphere will be affected by changes in the soil nutrient availability and accessibility. Plant-associated microbiota influence plant growth by supplying nutrients such as nitrogen, phosphorus, or potassium. On the other hand, availability of inorganic nutrients influences the root microbiota by affecting root secretions and exudates that provide easily degradable carbon for the heterotrophic bacteria (82). Additionally, drought stress increases osmotic pressure in the soil, resulting in reduced nutrient solubility and poorer diffusion. Thus, the most restrictive nutritional status would be represented in our low water and low nutrient (LW,LN) sample. Bacterial groups enriched in the LW,LN sample could be benefitting the host by solubilizing pools of limiting soil nutrients, such as phosphate, and transform it into bio-available forms, thereby increasing nutrient uptake and growth. It is possible that cogongrass's ability to recruit and maintain the microbial flora under drought, including a number of unique groups representing Proteobacteria, Actinobacteria, and single species from Bacteroidetes (*Parafilimonas terrae*) and Chloroflexi (*Ktedonobacter racemifer*), can be contributing to its remarkable success as an invasive plant. Both *P. terrae* and *K. racemifer* are aerobic and non-motile, and were shown to have a moderate tolerance to salinity (able to grow in the presence of up to 1.5% NaCl) (81, 83), which could possibly provide them with a metabolic advantage under drought conditions. Interestingly, an Actinobacteria species *Amycolatopsis sacchari* showed a high growth preference for LW,HN, the most osmotically challenging conditions tested in our experiment. *A. sacchari* and a number of other species of the genus *Amycolatopsis* were reported to be able to grow in presence of 5% NaCl (84).

Important gene families and their functions responding to water and nutrient stress

The overarching trend, based on the CAZy (Carbohydrate-Active enZymes) Database, was a striking induction of cell wall, membrane and desiccation-related genes in both drought-subjected samples (LW,LN and LW,HN), and a marked downregulation in regularly-watered samples (HW,LN and HW,HN). Our findings are in agreement with the notion that drought within the microbiome community causes a general response focused on thickening and remodeling their cell walls. The primary responses within the

cell wall for both Gram-positive and Gram-negative bacteria are aimed at changes in architecture and composition to create a more hydrated microenvironment, facilitating biofilm formation and better protection from desiccation (85). Consistent with this concept, we noted an abundance of enzymes involved in the metabolism of N-Acetylglucosamine (GlcNAc) in low water treatments. GlcNAc is an important component of the cell wall peptidoglycans in both Gram-positive and Gram-negative bacteria (86). In addition, glycosyltransferases N-acetylglucosaminyltransferase and N-acetylgalactosaminyltransferase are involved in the conversion of UDP-GlcNAc in the biosynthesis of lipid A of the lipooligosaccharide (LOS), a low-molecular-weight form of lipopolysaccharide (LPS) that is the major component of the outer membrane of Gram-negative bacteria. LPS defines the structural integrity of the bacterial cell wall, protects their membranes from chemical attack and helps stabilize the overall membrane structure. Additionally, we identified cellulose synthase in low water treatments, which is an enzyme responsible for the formation of cellulose microfibrils that are made on the surface of cell membranes to reinforce cells walls, and have been shown to participate in bacterial biofilm formation (87). Intriguingly, we also discovered chitin synthase and chitin oligosaccharide synthase, which is particularly interesting given that these are not *bona fide* bacterial genes, and have been acquired by microbes from eukaryotic donors via Horizontal Gene Transfer. Chitin, a polymer of GlcNAc, can contribute to the rigidity and integrity of cells.

Our analysis also uncovered several enzymes related to sucrose metabolism that responded to drought. Trehalose phosphorylase is an enzyme that catalyzes a reversible conversion of trehalose, a disaccharide formed by two glucose molecules, which is well known as an osmoprotecting compatible solute accumulated in bacterial cells in response to dehydration stress (88). Additionally, we detected sucrose synthase and sucrose-phosphate synthase, which catalyze two reversible steps in sucrose biosynthesis. Along with trehalose and several other disaccharides, sucrose can serve as an osmoprotectant in bacterial cells (89). Enzymes implicated in the modulation of cell wall surface include hyaluronan synthase, which forms hyaluronic acid by polymerization of glucuronic acid with GlcNAc. Hyaluronan is a polysaccharide with an exceptional molecular weight that has unique capacity in retaining water, and exerts excellent protection to the bacterial cells under drought stress (90). Moreover, bacterial membranes also adapt to water deficit as means to counter higher osmotic pressures (91). We found an overrepresentation of enzymes related to phosphatidylinositol metabolism, which could likely modify the structure and fluidity of bacterial membranes.

Limitations And Future Directions

Our work with a common garden experiment advances earlier understanding of the interactions among invasions, drought and nutrient addition, and will provide a deeper understanding of invasion mechanisms. Therefore, future work should compare the consistence with long-term field observation, and also consider capturing the time series change of microbial composition and function. In addition, this study gives some hints that invasive grass may have higher drought resistance over time due to the plant-microbe interaction. Therefore, it is critical to consider the role of soil microbes into modeling and predictions of plant invasion, and future invasion might be even severe due to the plant-microbe

interaction. Lastly, it would be worthwhile to explore competition process between native and invasive species by incorporating microcosm and field experiments to understand the effect of soil microbial communities on plant drought resistance.

Conclusion

In conclusion, our research indicates that the associated microbial communities may have a potential long-term impact on increasing invasive grass drought resistance, ultimately, future invasion might be severe due to the plant-microbe interaction than our current prediction. As a result, the role of microbiomes really needs to be included in current modeling practice, otherwise, lacking comprehensive consideration of the plant-microbe interaction, could lead to a significant underestimate of predictions of future invasion patterns. Additionally, including plant-microbe interaction in the model has been challenging due to the lack of fundamental understanding on microbiomes' impact and function. Hence, studies, like ours, will provide mechanistic understanding that contributes to future modeling studies.

Methods And Materials

Plant material

Cogongrass (*Imperata cylindrical* [L.] P. Beauv.) is a warm-season, perennial C₄ grass that negatively affects the agriculture and forestry industry (30) and is a substantial threat to biodiversity and ecosystem functions (31) in its invasive range. Cogongrass, as a C₄ grass, has shown to ameliorate water stress in the drought treatment (32) due to its general higher water use efficiency than C₃ grass (33), or the reduced soil surface temperature and increased humidity (32). This grass, which spreads via both seeds and rhizomes (34), is native to east Asia, but was introduced to south Alabama in the early 1910s and again in the mid-1940s (35). Subsequent introductions into Florida, Mississippi and Texas occurred during the 1920-40s for forage testing purposes (36). Considering Florida as an example, the economic analysis revealed that cogongrass resulted in economic losses throughout Florida of \$35 million annually to the forestry supported business sectors (37).

Experimental design

We collected planted cogongrass from the Entomology & Nematology Department greenhouse at the University of Florida in Gainesville, FL, then transferred them to the greenhouse at the Institute of Food and Agricultural Sciences at the University of Florida in Davie, FL. All the cogongrass belonged to a same genotype that was originally collected in Florida and they grew in similar environments before (38). Cogongrass rhizome samples were collected from the field then relocated to the greenhouse in IFAS. Additionally, based on a genotyping-by-sequencing approach to identify genetic diversity of cogongrass in the south-eastern United States, Burrell, *et al.* (39) found each of the four clonal lineages of cogongrass was highly homogeneous and cogongrass has limited evolutionary potential. Therefore, the cogongrass lineage we used in this study could represent, at least, a main part of natural cogongrass populations.

Cogongrass was grown for two months to allow acclimation to the new environment prior to use in this study. Single- cogongrass rhizome fragments (≈ 15 cm in length with at least four nodes) were cut from the acclimated cogongrass and then was transplanted horizontally into pots (25 cm diameter $\times$ 23 cm height) filled with commercial potting mix (Fafard[®] Professional Growing Mix, Sun Gro[®] Horticulture Canada Ltd., Agawam, MA). We watered all the pots until saturate weekly for a month and selected all the pots with successfully rhizome germination for later experiments. During both plant propagation and the experiment (September 2018 to March 2019), plants were grown at 25–30 °C in the Davie, FL greenhouse which mirrored natural conditions in the area.

This experiment was conducted with a full factorial design manipulating water availability (low and high) and nutrient concentration (low and high). To manipulate water availability, half the plants were water to saturation once a month for low water treatment and half were watered once every two weeks in high water treatment. The watering frequency was determined in accordance with watering periods used in Webster and Grey (40), which resulted in similar soil water contents that Burns (7) used in her study. For the nutrient manipulation, the low nutrient treatment was not fertilized during the experiment while the high nutrient treatment was fertilized every two weeks. Fertilizer was purchased from General Hydroponics (genhydro Inc, CA, USA) and was dispensed into each pot according to the manufacturer's recommendations (Total Nitrogen: 0.009%; P₂O₅: 0.007%; K₂O: 0.014%). Each of the four treatment combinations was replicated 11 times (total of 44 pots) and all pots were placed in a randomized complete block design in the greenhouse and monitored between September 2018 and March 2019.

Soil sampling and DNA extraction

Prior to the experiment, we collected three soil samples of the original potting mix we used for soil bacterial (16S rRNA gene) and fungal (internal transcribed spacer ITS) sequencing to have the information of the initial bacterial and fungal communities (Fig. S1 and S2 in the Appendix). After the experiment, rhizosphere soil samples were obtained from 11 disease- and pest-free plants in each treatment combination. We separated soil from plants roots, focusing on a soil within 2-mm of the root surface using a combination of gentle shaking and removal with sterile brushes. Samples of soil from the four corners of each pot were also collected and pooled. All soil samples were aseptically sieved (2 mm) in the greenhouse and transported to the laboratory in centrifugal tubes on ice, then stored at -20 °C within 30 minutes since the samples were collected.

From each treatment combination, we randomly selected eight biological replicates from the 11 rhizosphere samples (each from the rhizosphere of a single plant), generating a total of 64 samples. Total DNA was extracted from 250 mg of each sample using a using the DNeasy Power Soil Kit (Qiagen, Valencia, CA, USA) according to the low microbial biomass method provided by the manufacturer. DNA concentration was measured using Qubit[®] dsDNA Assay Kit in Qubit[®] 2.0 Fluorometer (Life Technologies, CA, USA). The purity of DNA was expressed as the ratio of absorbance at 260 nm and 280 nm (A_{260}/A_{280}) using a Nanodrop[®] spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA

degradation degree and potential contamination were assessed on 1% agarose gels. According to the concentration, DNA was diluted to 1 ng/μL using sterile water.

For each pot we obtained a single DNA sample, which was used for soil bacterial (16S rRNA gene) and fungal (internal transcribed spacer ITS) sequencing. 16S rRNA and ITS genes of distinct regions (16S V4 and ITS1) were amplified using specific primer (16S V4: 515 F (5'-GTGCCAGCMGCCGCGG-3') and 806 R (5'-GGACTACHVGGGTWTCTAAT-3') (41), ITS 1-5: (F: 5'-GCTGCGTTCTTCATCGATGC-3', R: 5'-GGAAGTAAAAGTCGTAACAAGG-3') (42)) with the barcode. All PCR reactions were carried out with Phusion® High-Fidelity PCR Master Mix (New England Biolabs). Mix same volume of 1×loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with GeneJET™ Gel Extraction Kit (Thermo Scientific). Sequencing libraries were generated using Ion Plus Fragment Library Kit 48 rxns (Thermo Scientific) following manufacturer's recommendations. The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo Scientific). Finally, the libraries were sequenced on the IonS5™XL platform and 400 bp/600 bp single-end reads were generated.

16S rRNA gene and ITS sequencing data processing

Quality filtering of the raw reads were performed under specific filtering conditions to obtain the high-quality clean reads according to the Cutadapt (43) (V1.9.1, <http://cutadapt.readthedocs.io/en/stable/>). The reads were compared with the reference database (16s:Silva database, <https://www.arb-silva.de/>; ITS: Unite database, <http://unite.ut.ee/>) (44, 45), using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) (46) to detect chimera sequences (47), and then the chimera sequences were removed. Operational taxonomic units (OTUs, cutoff 97% sequence identity for 16S rRNA and ITS) were clustered by UPARSE software (Uparse v7.0.1001, <http://drive5.com/uparse/>) (48). The phylogenetic taxonomy was assigned according to the Ribosomal Database Project (RDP) classifier at an 80% confidence threshold (Version 2.2) (49) using the Silva and UNITE databases for bacteria and fungi, respectively. α-diversity were calculated in QIIME software (Version 1.7.0) for each sample's fungal and bacterial community using the metrics of species richness (OTU number), Chao1, Shannon and Simpson index, ACE and Good's coverage. Chao1 and ACE indices were selected to identify community richness; Shannon and Simpson indices were used to identify community diversity; Goods coverage was to characterized sequencing depth.

Shotgun metagenome sequencing and data processing

Twelve samples (three per treatment combination) were randomly selected for shotgun metagenome sequencing. Using 1 μg DNA per sample as input material, libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit (NEB, USA), and index codes were added to attribute sequences to each sample. Libraries were quantified by fluorometry, immobilized and processed onto a flow cell with a cBot (Illumina, San Diego, USA) followed by sequencing-by-synthesis on Illumina HiSeq platform (100bp Paired-end, Illumina, San Diego, USA). Preprocessing the raw data obtained from the Illumina HiSeq

sequencing platform using Readfq (V8, <https://github.com/cjfields/readfq>) was conducted to acquire the clean data for subsequent analysis. High-quality reads of each sample were assembled using the SOAPdenovo (v 2.04) assembler with the metagenomics model and default parameters (50). MEGAHIT software (v1.0.4-beta) could be used to assemble the Clean Data (51). We used MetaGeneMark (V2.10, <http://topaz.gatech.edu/GeneMark/>) to predict ORFs from the Scaffigs assembled from each sample as well as the Scaffigs from the mixed assembly. DIAMOND software (V0.7.9, <https://github.com/bbuchfink/diamond/>) was used to blast the unigenes to the sequences of bacteria, fungi, archaea, and viruses which were all extracted from the NR database (version: 20161115, <https://www.ncbi.nlm.nih.gov/>) of NCBI with the standard parameter (52). Functional annotation of metagenomes was conducted using DIAMOND software (V0.7.9) to blast unigenes to CAZy database (version 20150704, <http://www.cazy.org/>) (53), and the Comprehensive Antibiotic Resistance Database (<https://card.mcmaster.ca/>) with the parameter setting of blastp, $\text{evalue} \leq 1 \text{e}^{-30}$ (54). As an algorithm for high-dimensional biomarker discovery and explanation of genomic features characterizing the statistically different among four groups, linear discriminant analysis (LDA) effect size (LEfSe) was employed to compare microbial communities and identify specific phylotypes of cogongrass rhizosphere responding to water and nutrient stress.

Statistical analysis

1. The effects of treatments on soil microbial diversity and abundance

Alpha diversity is applied in analyzing complexity of species for a sample through 6 indices, including Observed species, Chao1, Goods coverage, ACE, Shannon and Simpson. All these indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R software (Version 2.15.3). The R packages Stats was used to perform statistical analysis. Significant differences in alpha diversity and taxonomy or relative abundance of genes between groups were tested using the Wilcoxon rank-sum test.

2. Links between microbial communities and plant performance (Structure Equation Model)

We used a hypothesized basic structural equation model designed to compare the direct and indirect effects of abiotic factors (soil water and nutrient contents) and biotic factors (above-and below-ground productivity and below-ground nutrient contents) on soil microbial structure (microbial gene richness, microbial gene composition, microbial taxonomic richness, microbial taxonomic composition) (data were listed in Table1 in the Appendix). We used the Unigenes blast with extraction from the NCBI NR database of bacteria, fungi, and archaea sequence using Diamond software. We filtered the blast results using the algorithm of Least Common Ancestors (LCA). Based on the LCA annotation results and the gene abundance table, the abundance information of each sample in each classification level (Kingdom, Phylum, Class, Order, Family, Genus and Species) was obtained. For the abundance of a species in a sample, it was equal to the sum of the gene abundance annotated as the species. Based on the LCA annotation results and the gene abundance table, the gene number table of each sample in each classification level (Kingdom, Phylum, Class, Order, Family, Genus and Species) was obtained. For a species, the number of genes in a sample was equal to the number of genes with non-zero abundance in

the annotated genes of the species. Correlation paths were hypothesized based on previous studies (55, 56). A hypothesized diagram was shown in Fig. S3 in the Appendix. These variables were used in linear mixed effects models with block as a random effect in the piecewise SEM. The SEM analyses were carried out through AMOS software, version 22 (IBM—SPSS Inc., Chicago, IL, USA).

Abbreviations

the Institute of Food and Agricultural Sciences (IFAS)

Ribosomal Database Project (RDP)

Operational Taxonomic Unit (OTU)

Non-Redundant Protein Sequence Database (NR database)

linear discriminant analysis (LDA)

linear discriminant analysis effect size (LEfSe)

Least Common Ancestors (LCA)

Structural equation modelling (SEM)

Carbohydrate-Active enZymes (CAZy)

N-Acetylglucosamine (GlcNAc)

lipooligosaccharide (LOS)

lipopolysaccharide (LPS)

Declarations

Ethics approval and consent to participate: None.

Consent for publication: The publisher has the Author's permission to publish the relevant Contribution.

Availability of data and material: Raw amplicon sequence data related to this study are available in the NCBI Sequence Read Archive under Bioprojects PRJNA638299 (bacteria and fungi). Raw shotgun metagenomics data are available on Bioproject PRJNA644680.

Competing interests: None.

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Authors' contributions: BZ, YY, AH, JZ and JQ designed the study, BZ and YY conducted the experiments and analyzed the data, KP and MA contributed to analyze the sequencing data, BZ, YY, KP wrote the first draft and all authors contributed to revisions of the manuscript.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

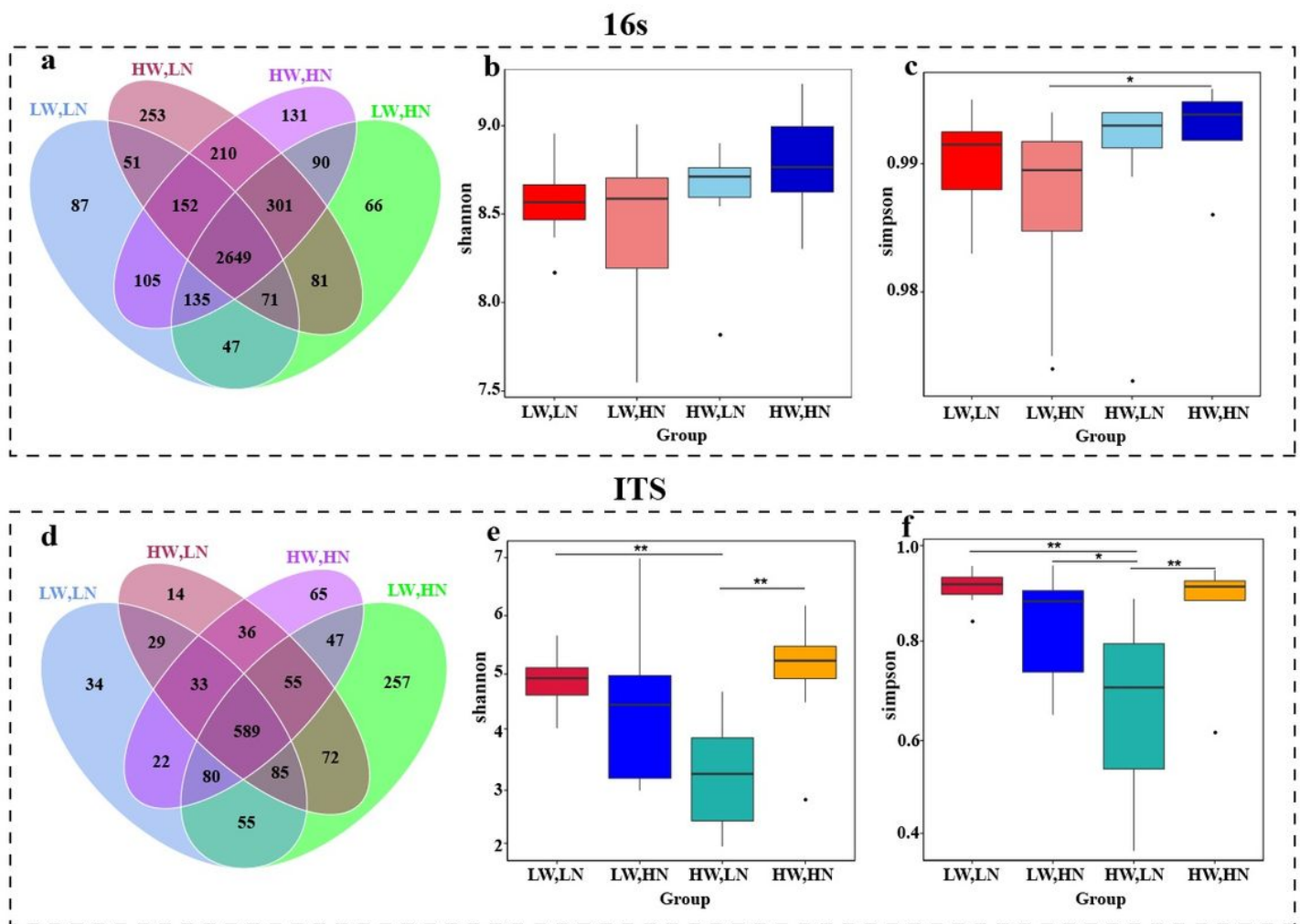


Figure 1

Venn diagram displaying unique and shared bacterial (16S rRNA) and fungi (ITS) OTUs in the four treatments (a and d), and α -diversity indices (Shannon and Simpson) in cogongrass rhizosphere soil (b-c and e-f). LW,LN: low water and low nutrient; LW,HN: low water and high nutrient; HW,LN: high water and low nutrient; HW,HN: high water and high nutrient. * $P < 0.05$, ** $P < 0.01$

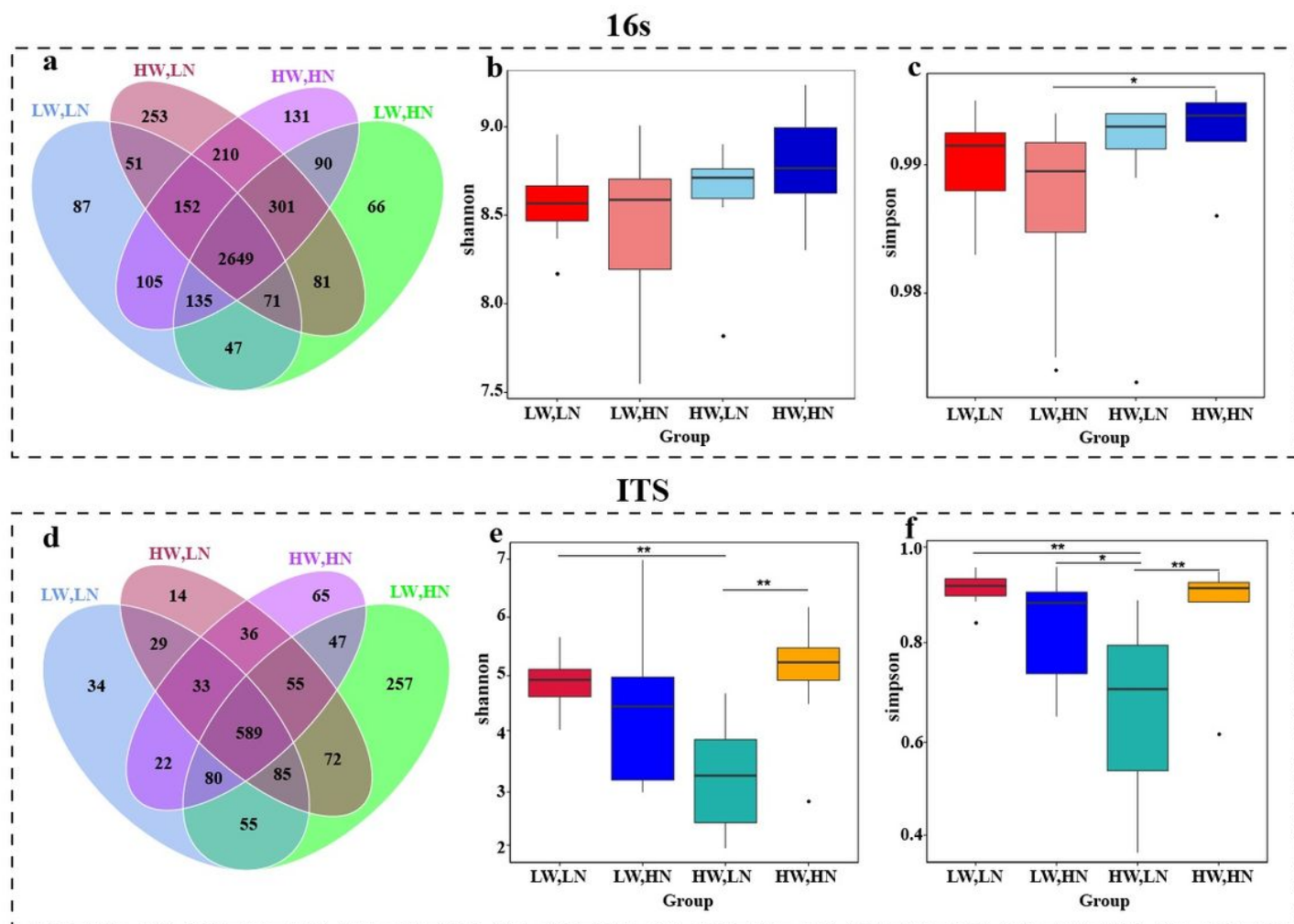


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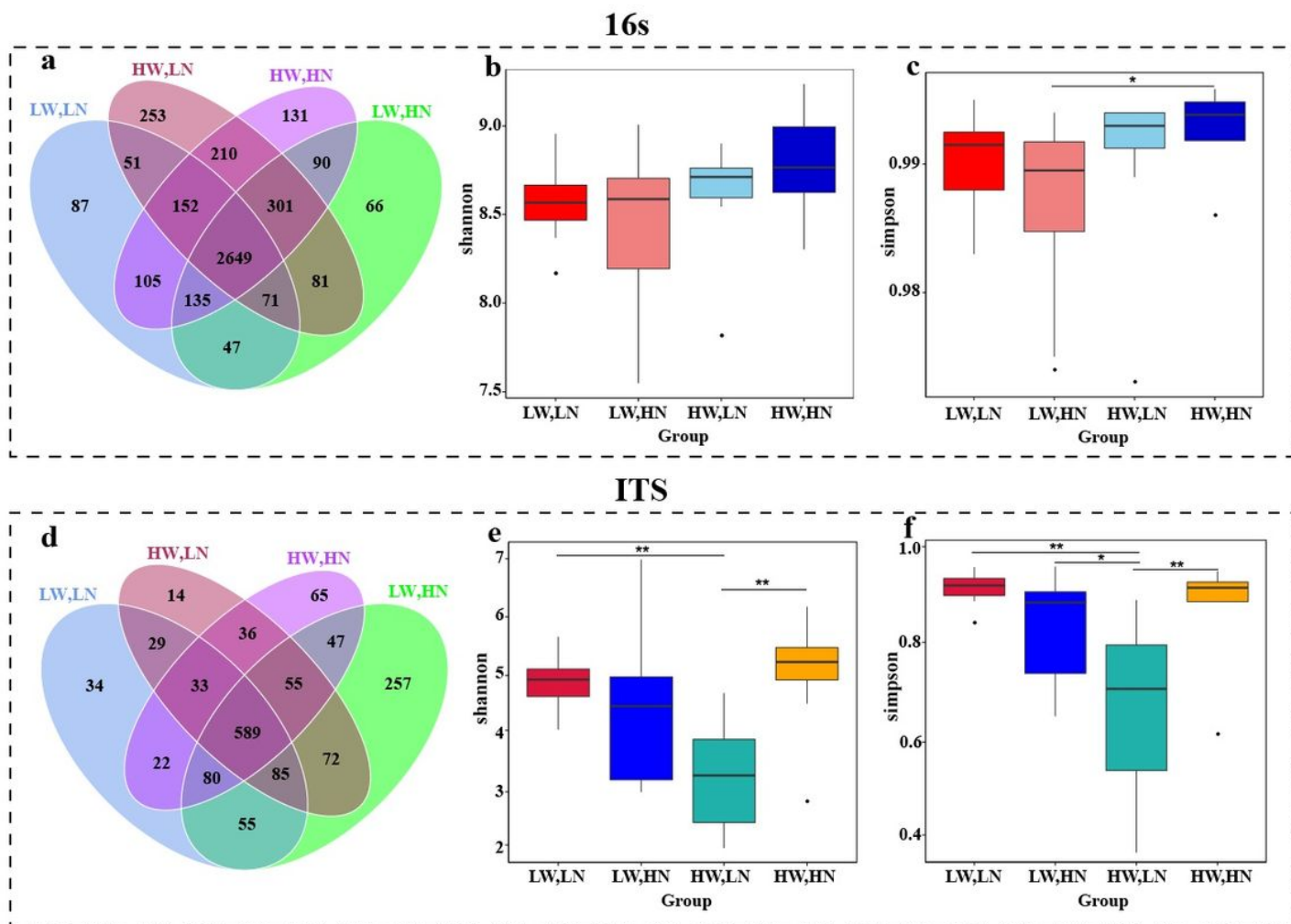


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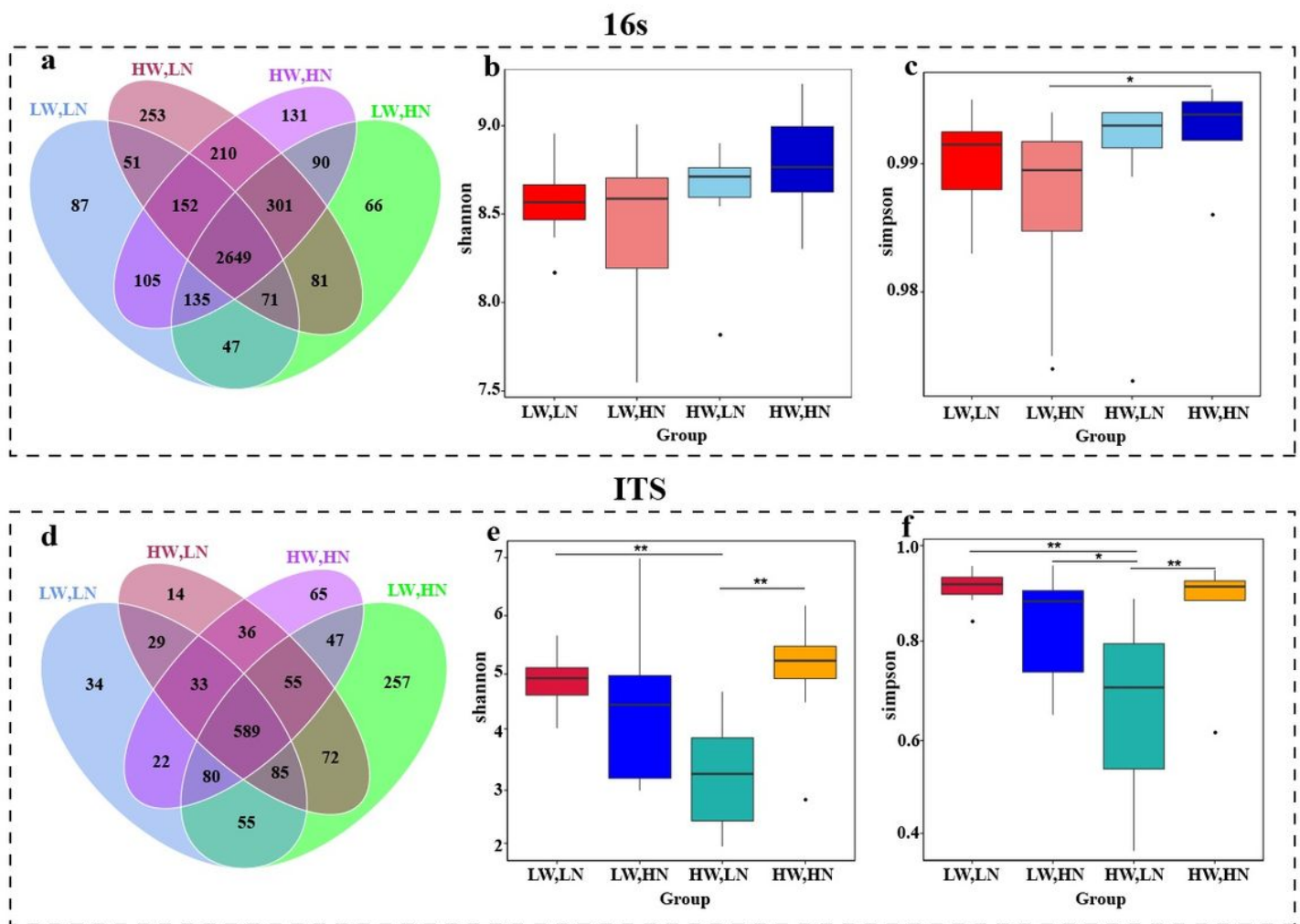


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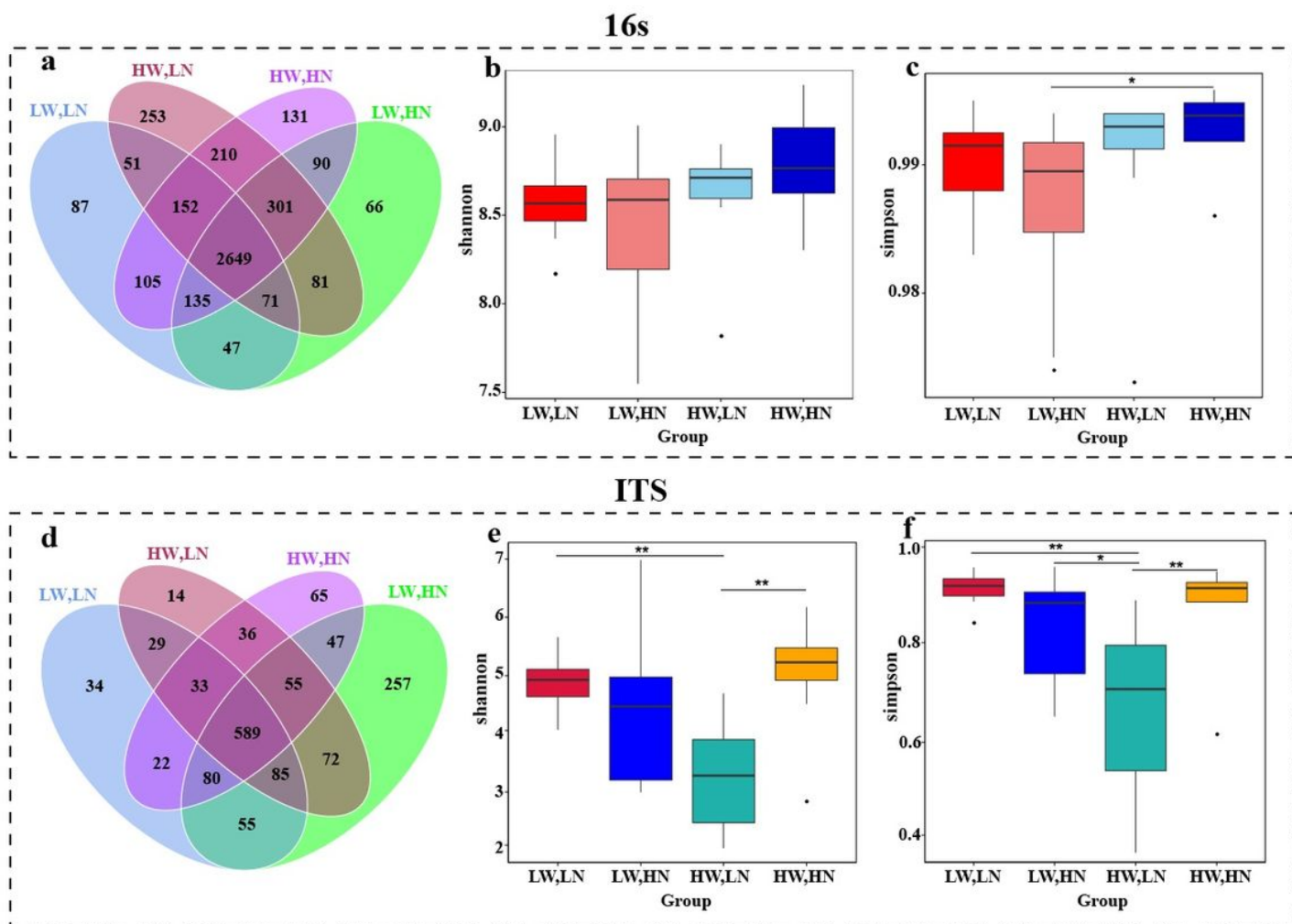


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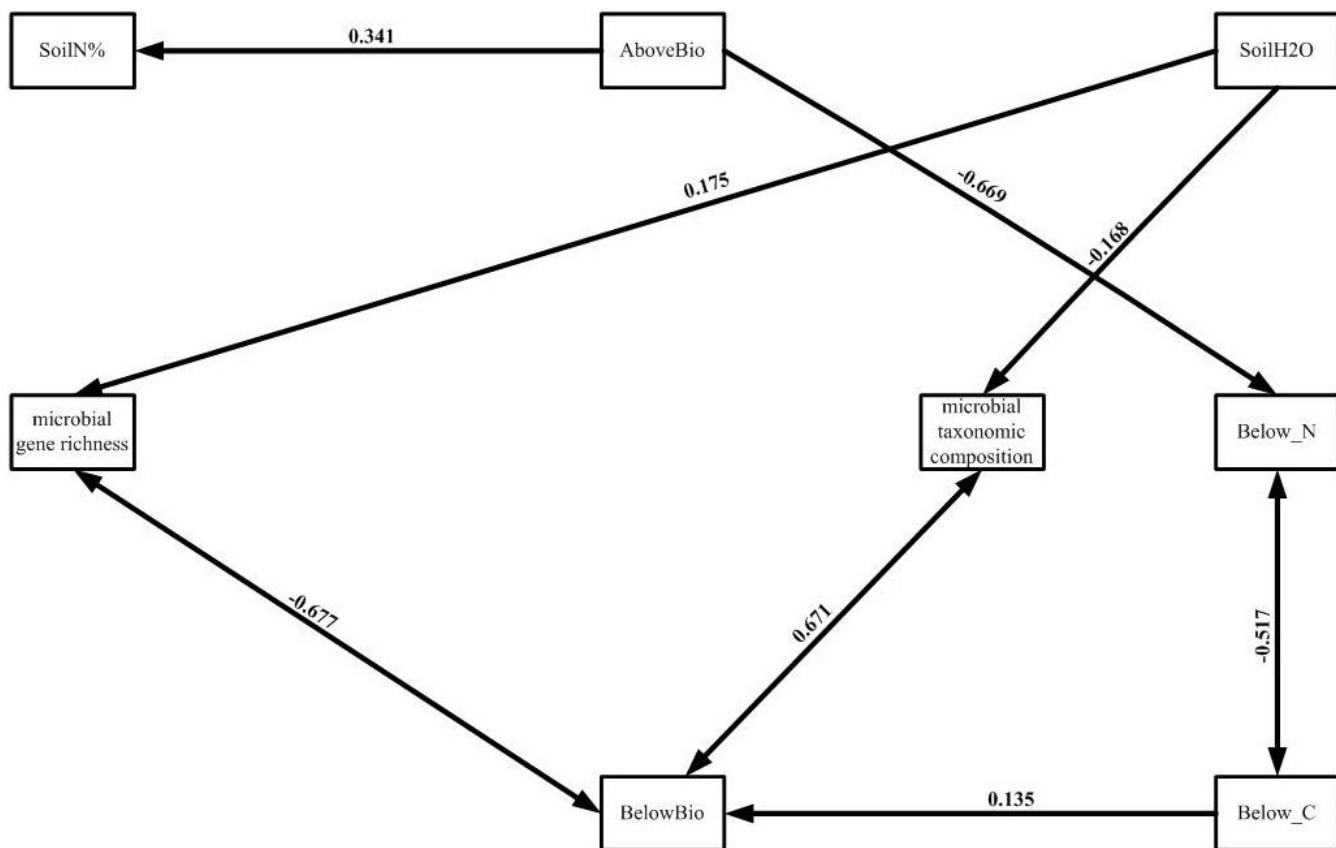


Figure 2

Structural Equation Model assesses the significant direct and indirect effects of the significant effects of abiotic factors (soil water (SoilH2O) and nutrient contents (SoilN%)) and biotic factors (above- (AboveBio) and below-ground productivity (BelowBio) and below-ground nutrient contents (Below_N and Below_C) on soil microbial structure (microbial gene richness and microbial taxonomic composition). Numbers indicate the strength and direction (i.e. positive vs negative) of the relationships.

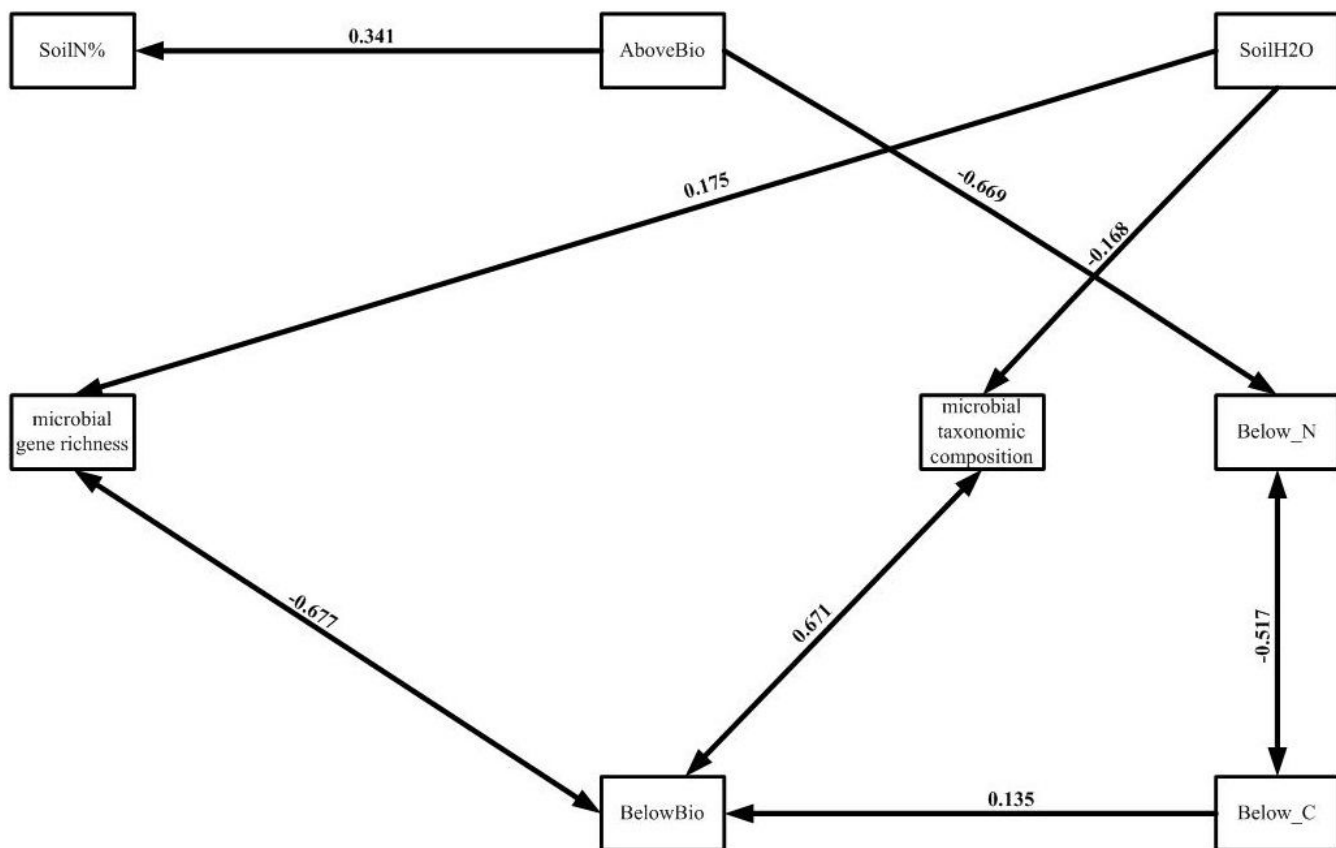


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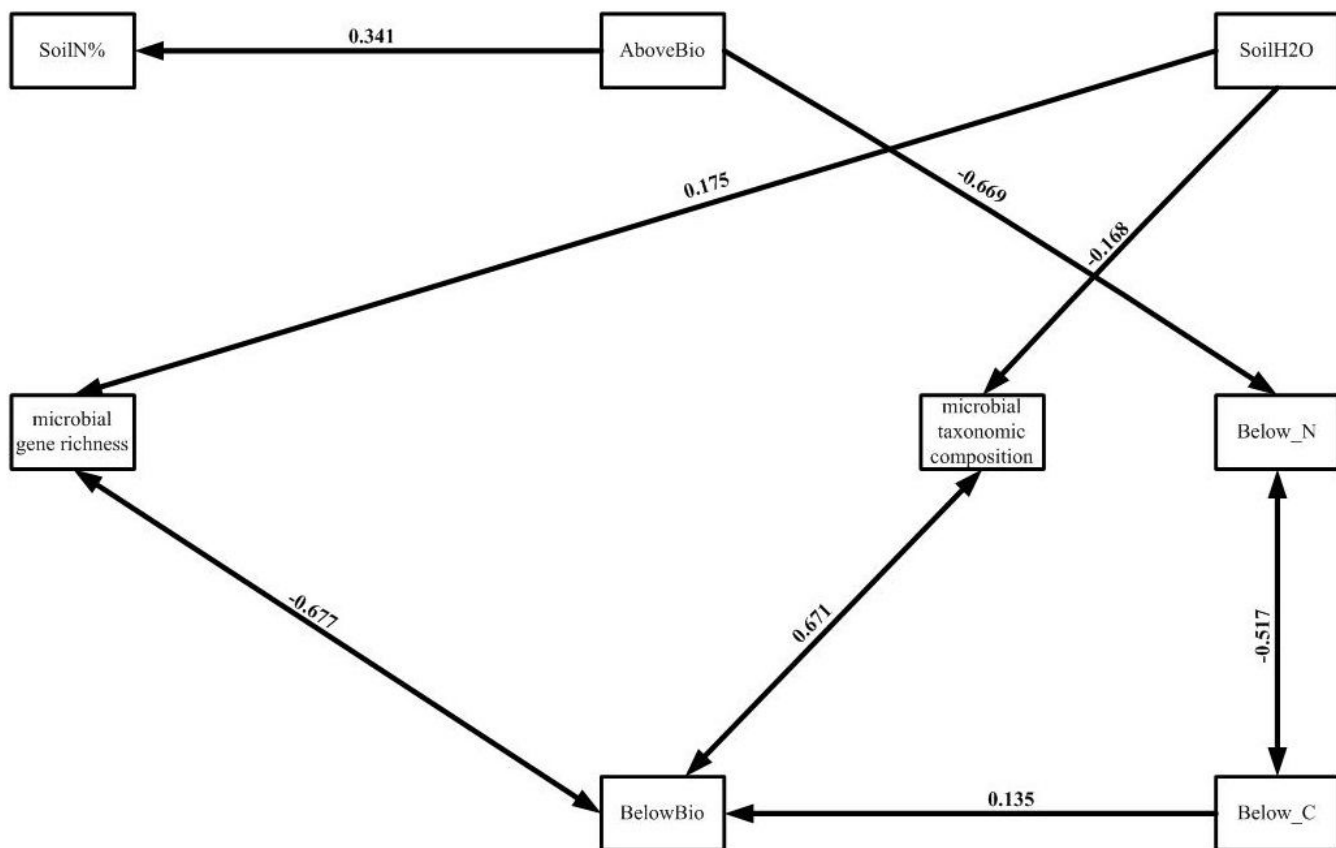


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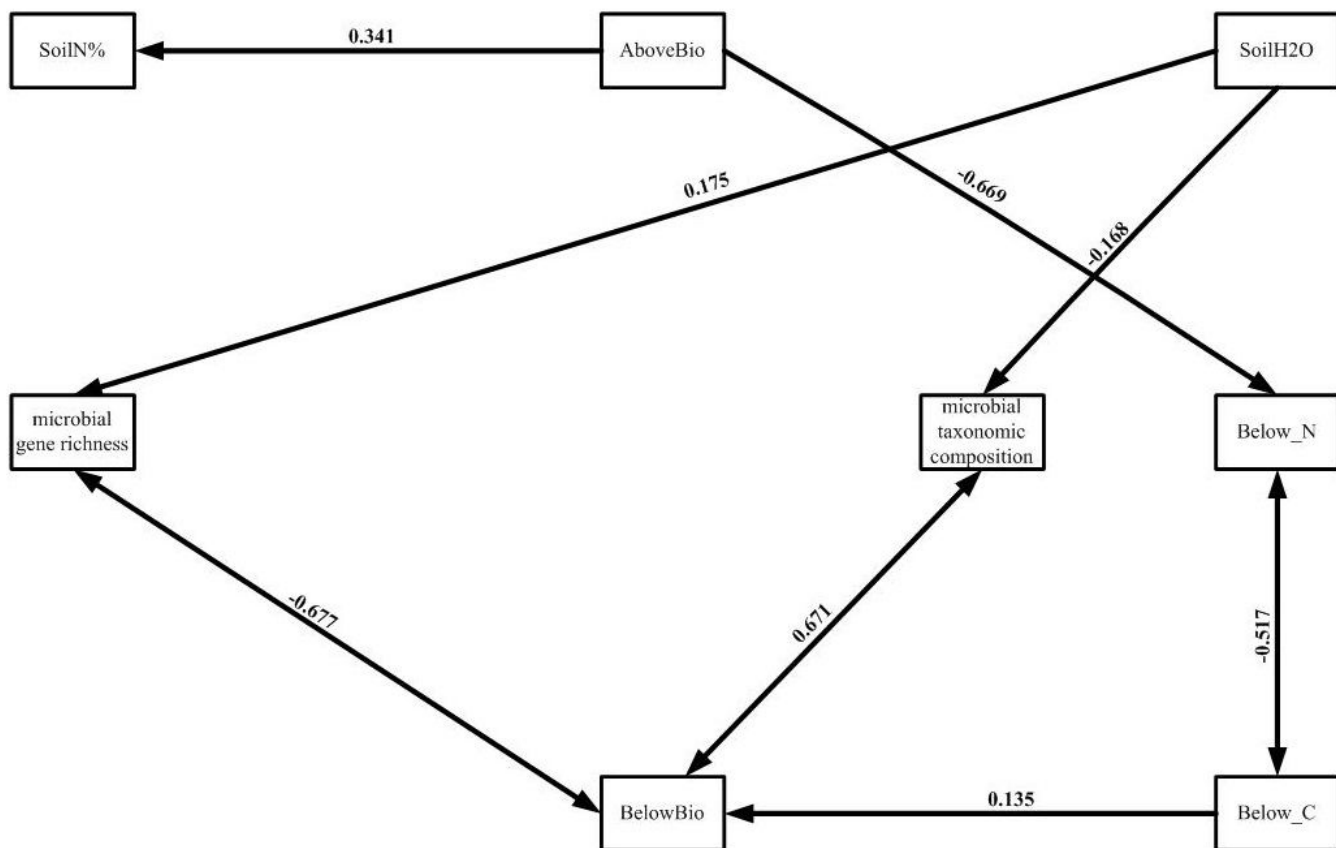


Figure 2

Structural Equation Model assesses the significant direct and indirect effects of the significant effects of abiotic factors (soil water (SoilH2O) and nutrient contents (SoilN%)) and biotic factors (above- (AboveBio) and below-ground productivity (BelowBio) and below-ground nutrient contents (Below_N and Below_C) on soil microbial structure (microbial gene richness and microbial taxonomic composition). Numbers indicate the strength and direction (i.e. positive vs negative) of the relationships.

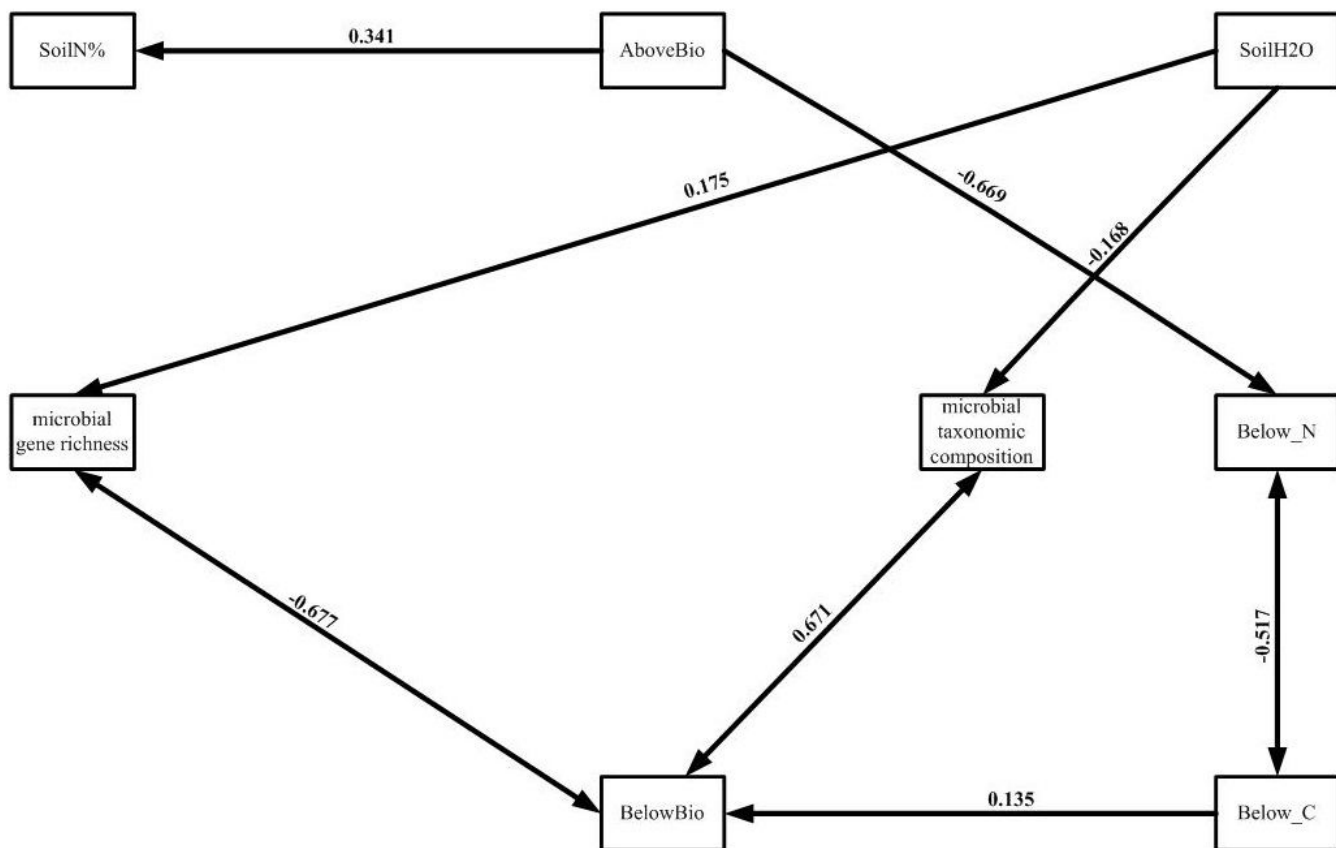


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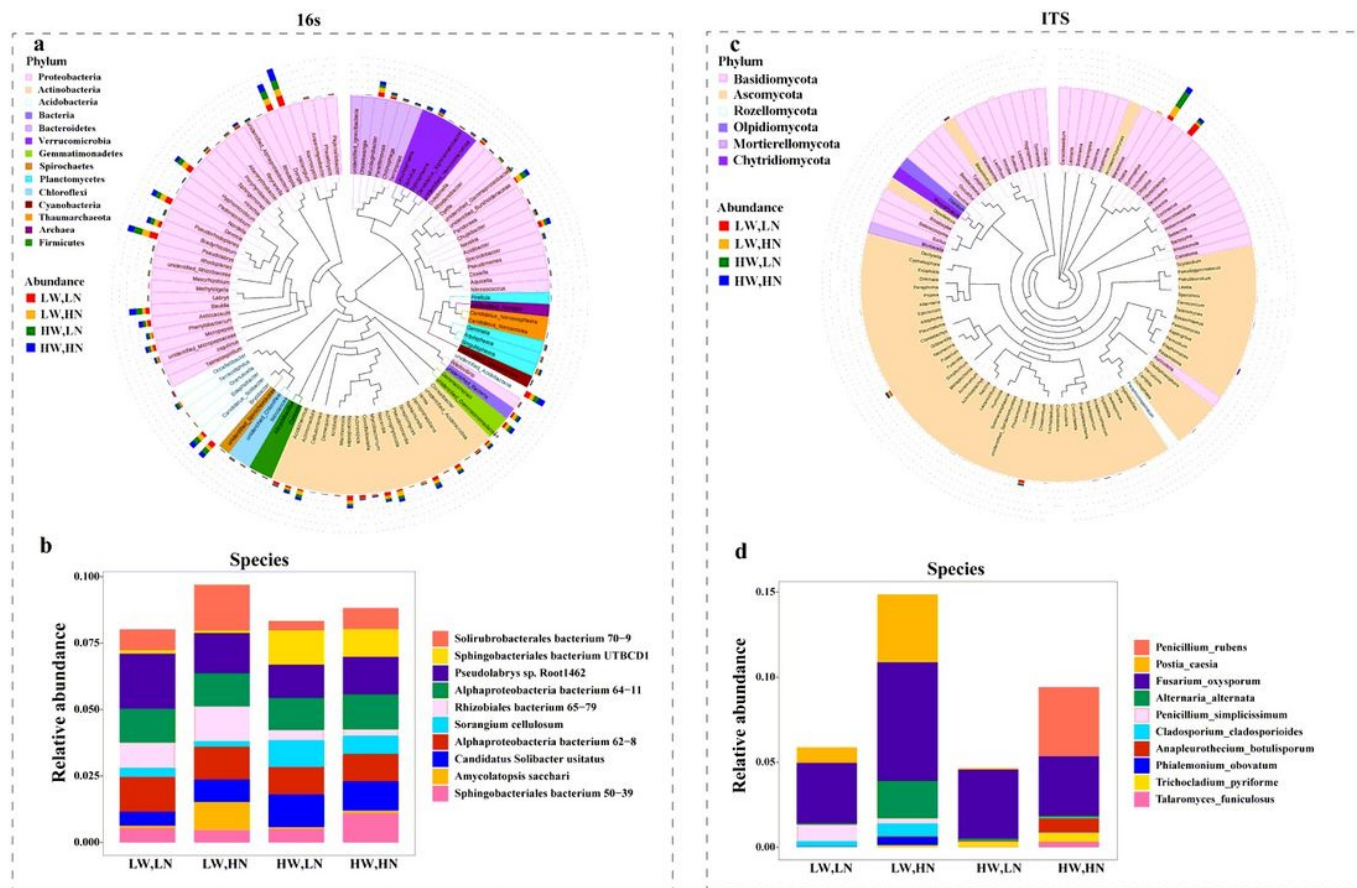


Figure 3

The Graphical Phylogenetic Analysis (GraPhlAn) of 16S (a) and ITS (c) in cogongrass rhizosphere soil. The GraPhlAn was constructed based on the phylogenetic tree of the microbes in all the samples. The rings around the phylogenetic tree are the microbial community of samples, which include four different treatments. The heatmaps are based on top 100 genus level. Bar plots show average relative abundances of the top bacterial species (b) and fungal species (d). LW,LN: low water and low nutrient; LW,HN: low water and high nutrient; HW,LN: high water and low nutrient; HW,HN: high water and high nutrient.

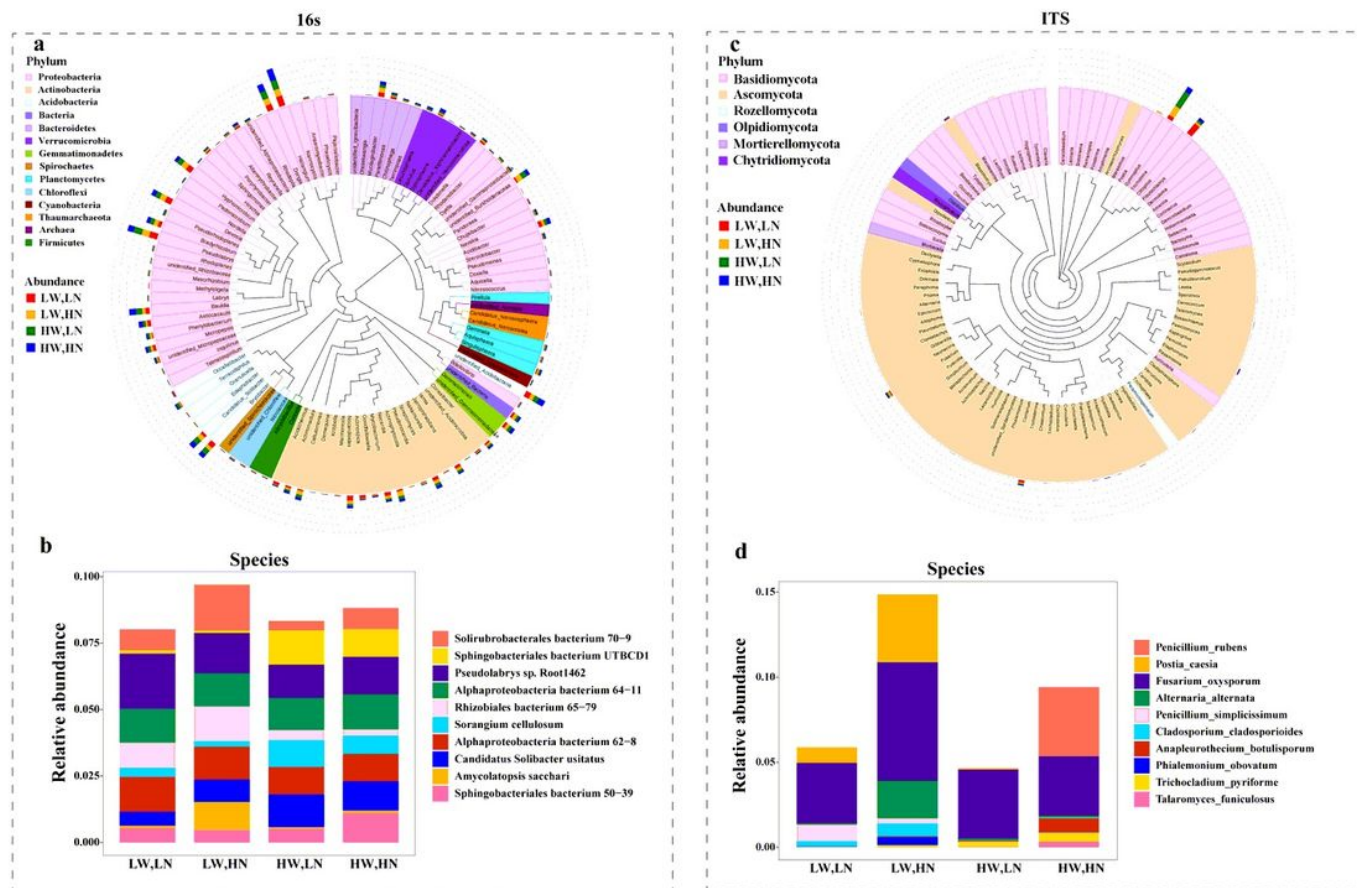


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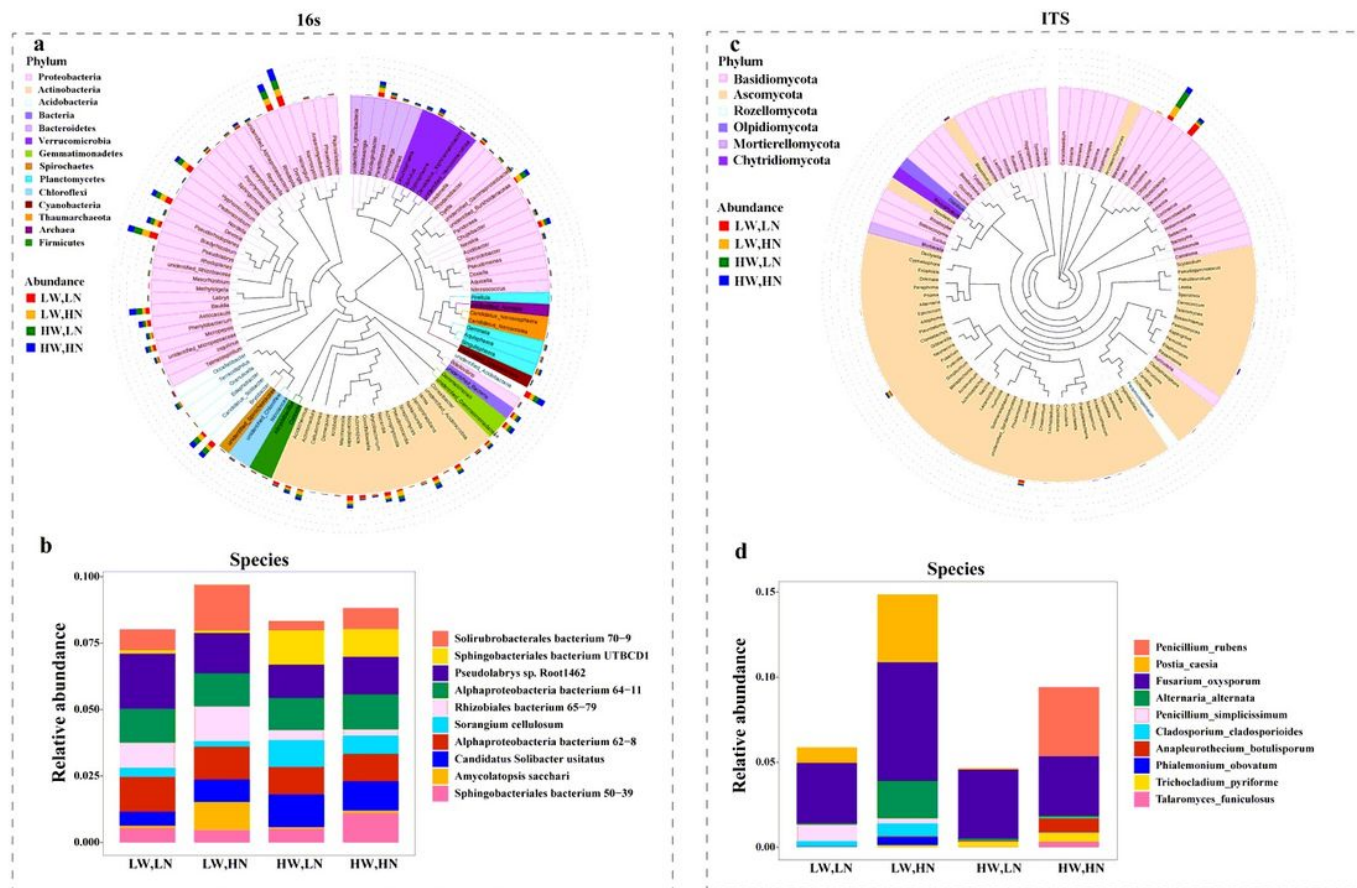


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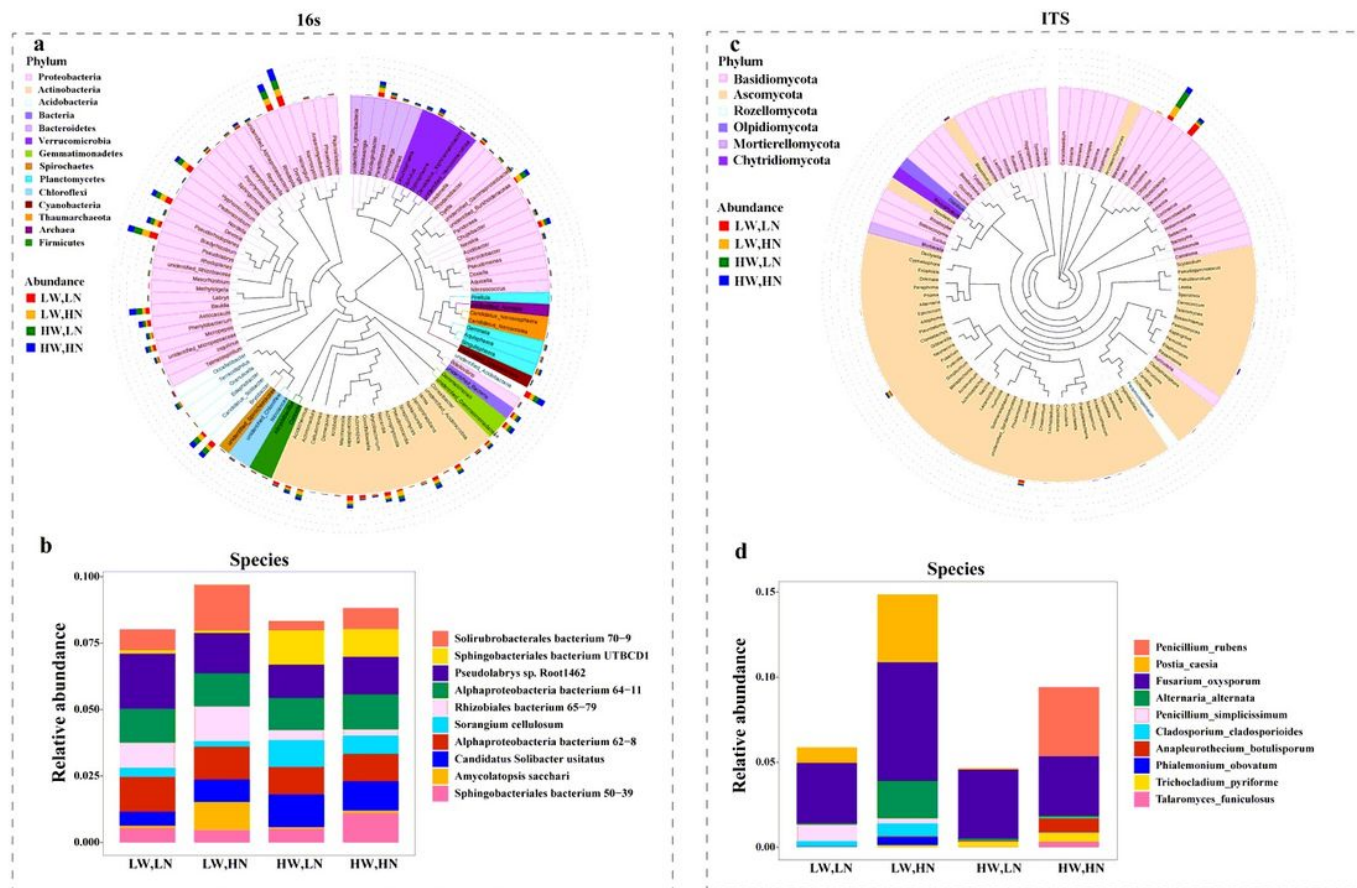


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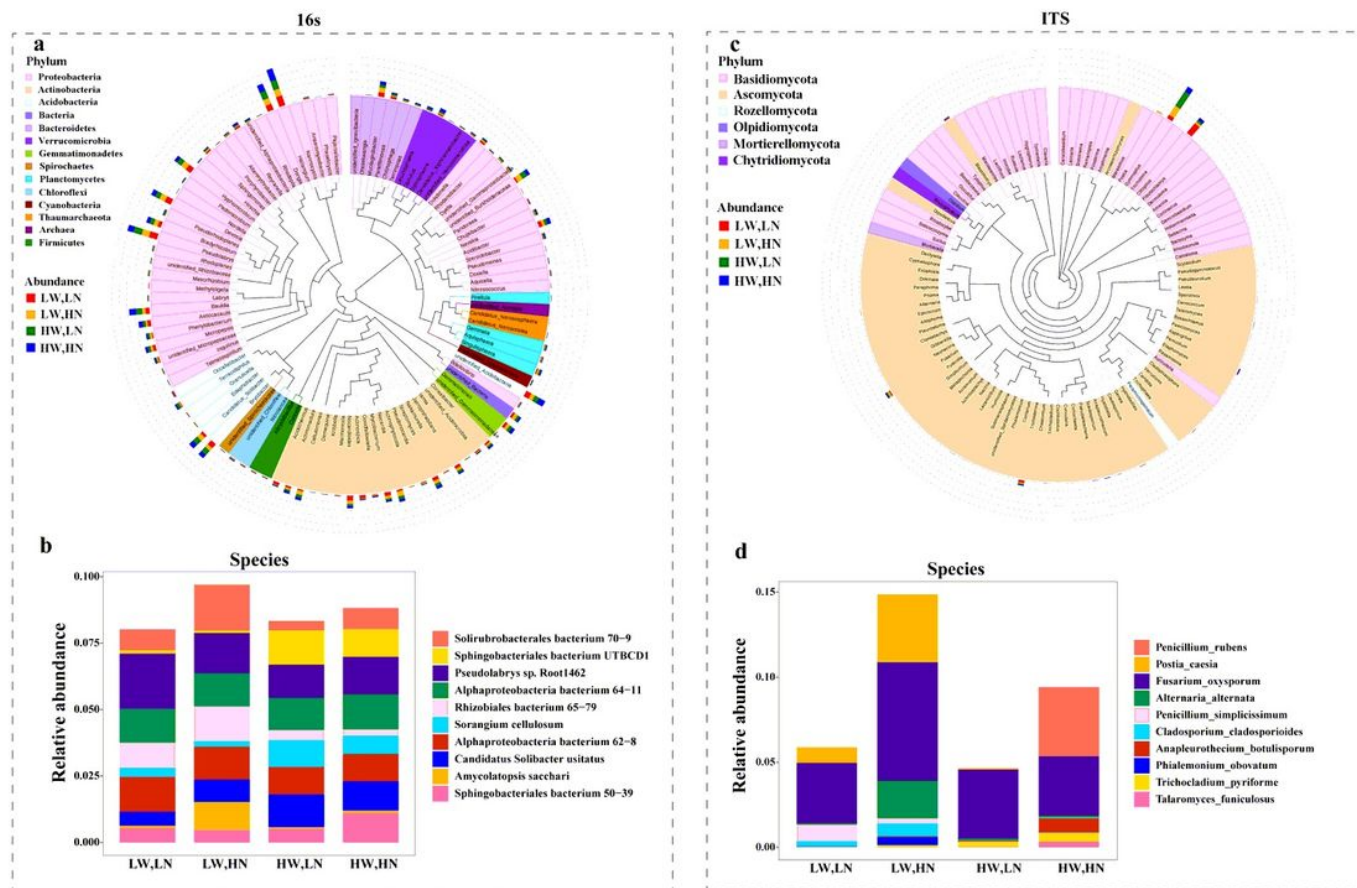


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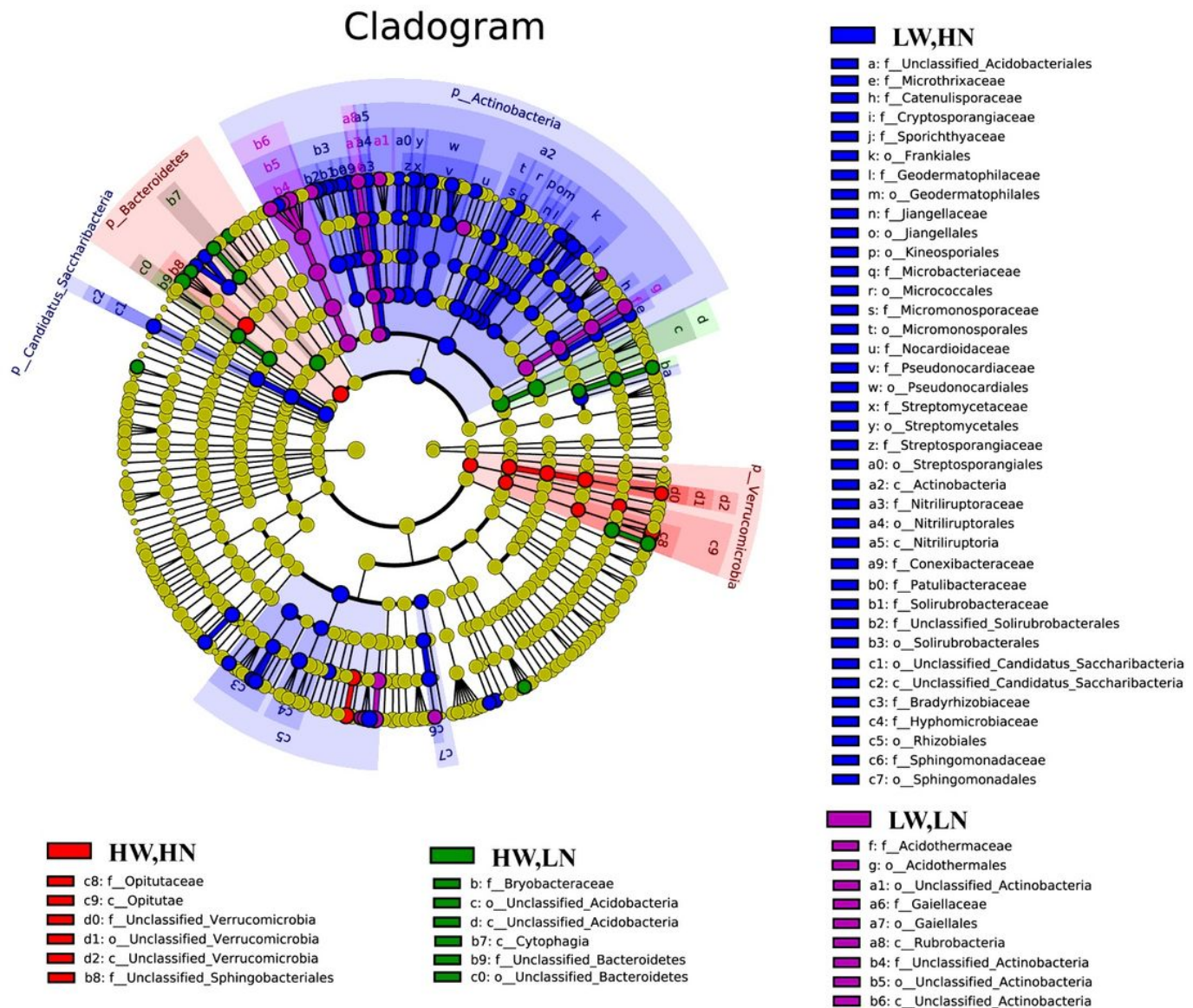


Figure 4

LEFSe analysis based on OTUs between four treatments. Cladogram indicating the phylogenetic distribution of bacterial lineages. The phylum, class, order, family, and genus levels are listed from inside to outside of the cladogram. The yellow circles represent the taxa with no significant differences. LW,LN: low water and low nutrient (purple); LW,HN: low water and high nutrient (high); HW,LN: high water and low nutrient (green); HW,HN: high water and high nutrient (red).

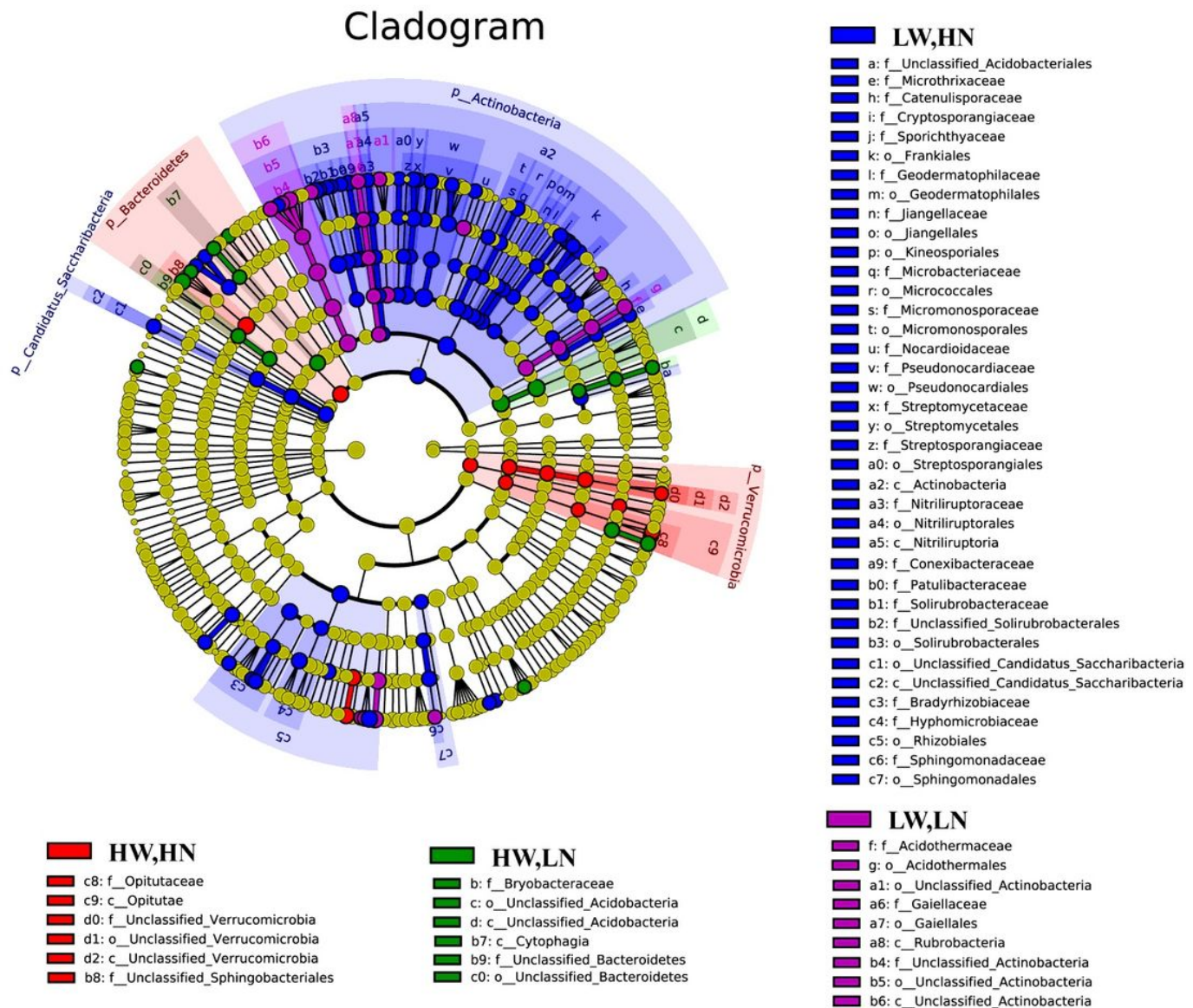


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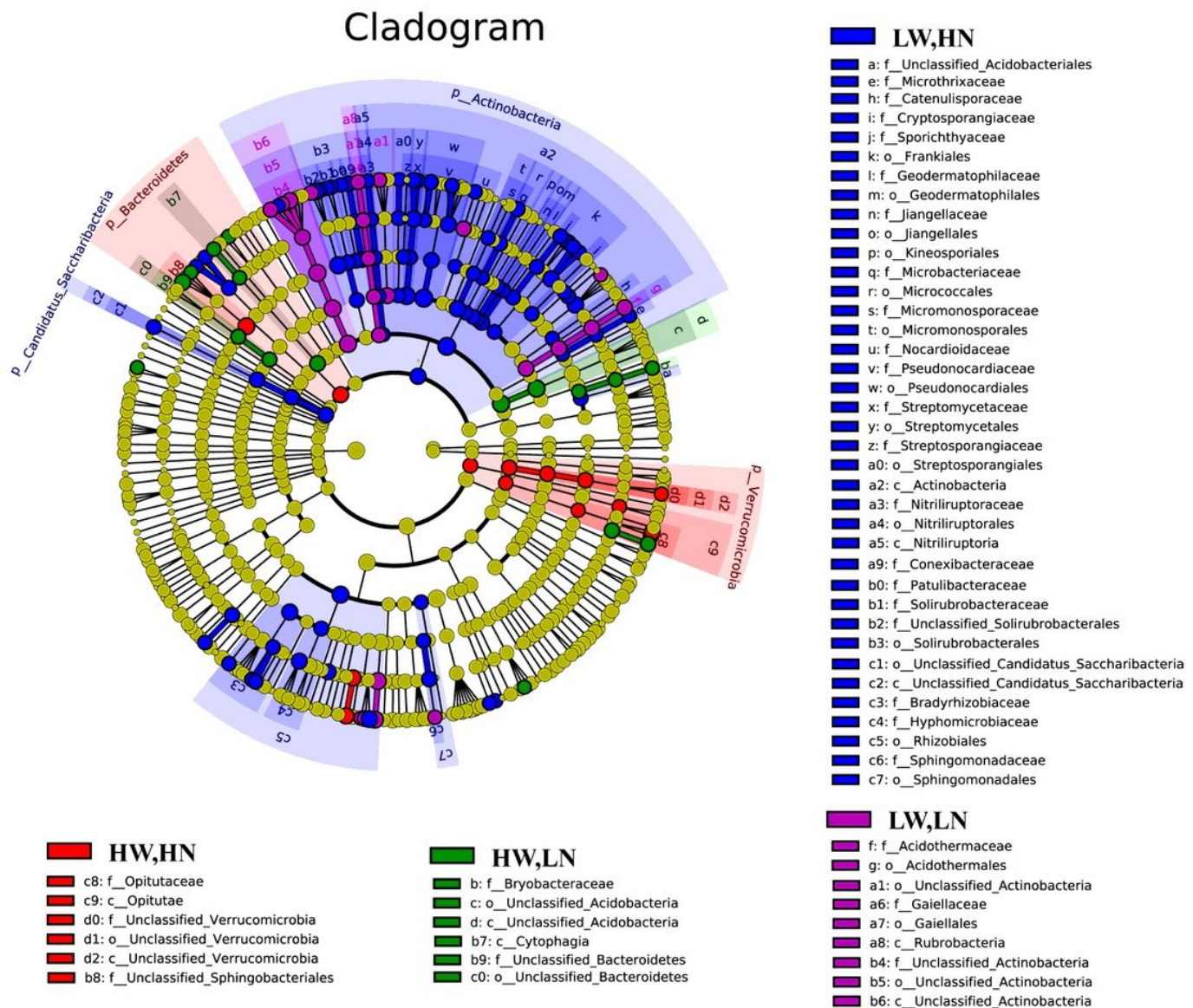


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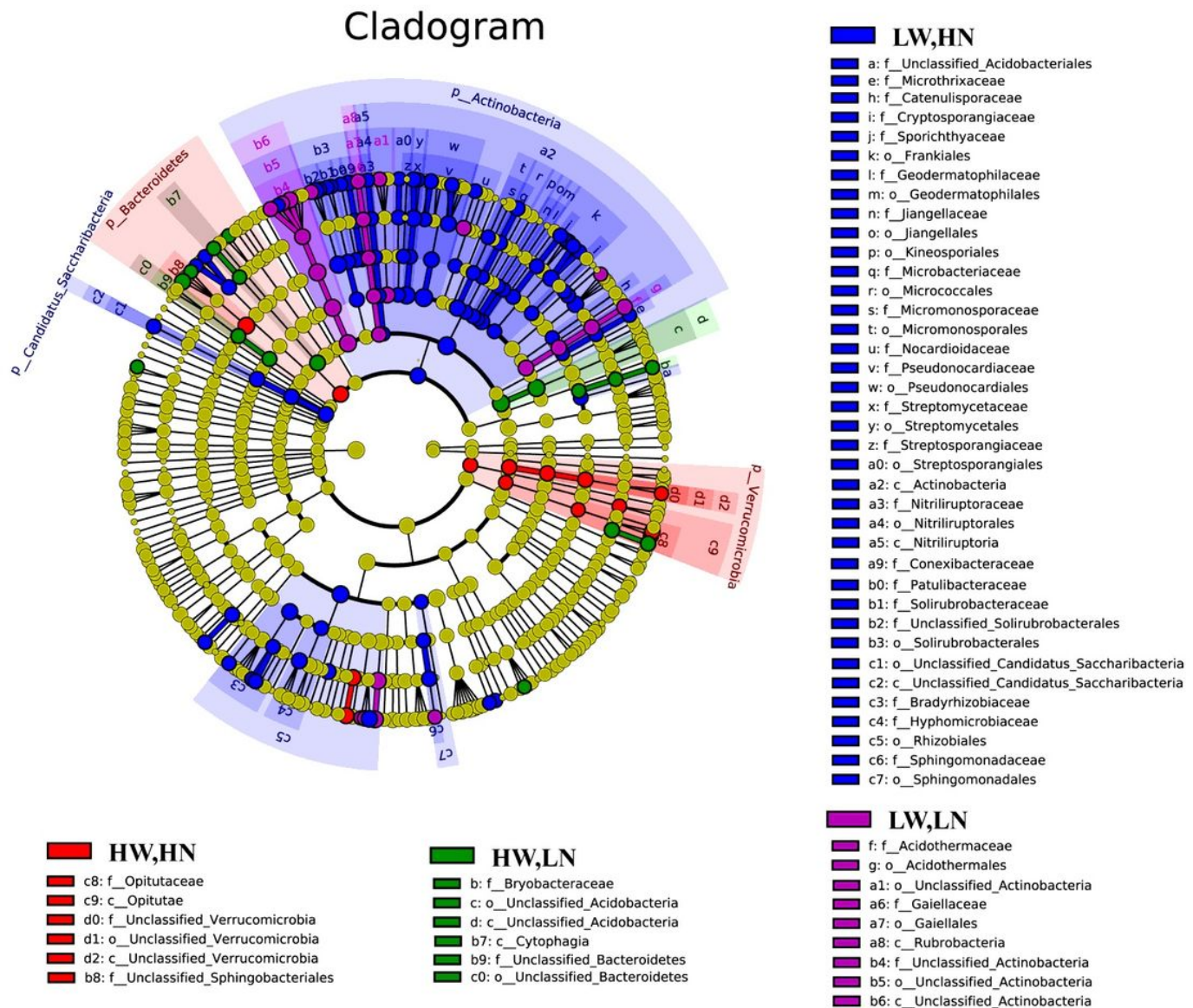


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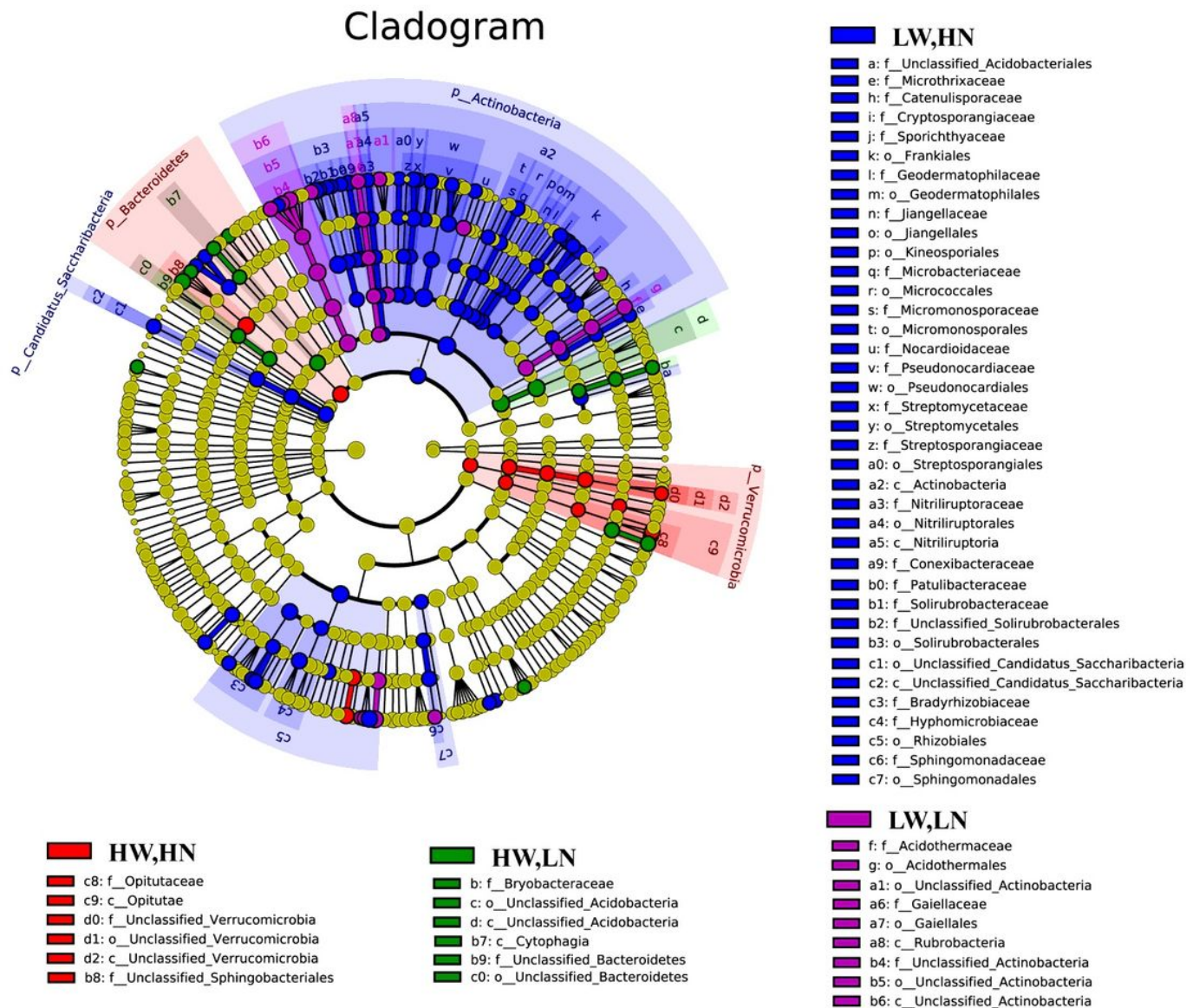


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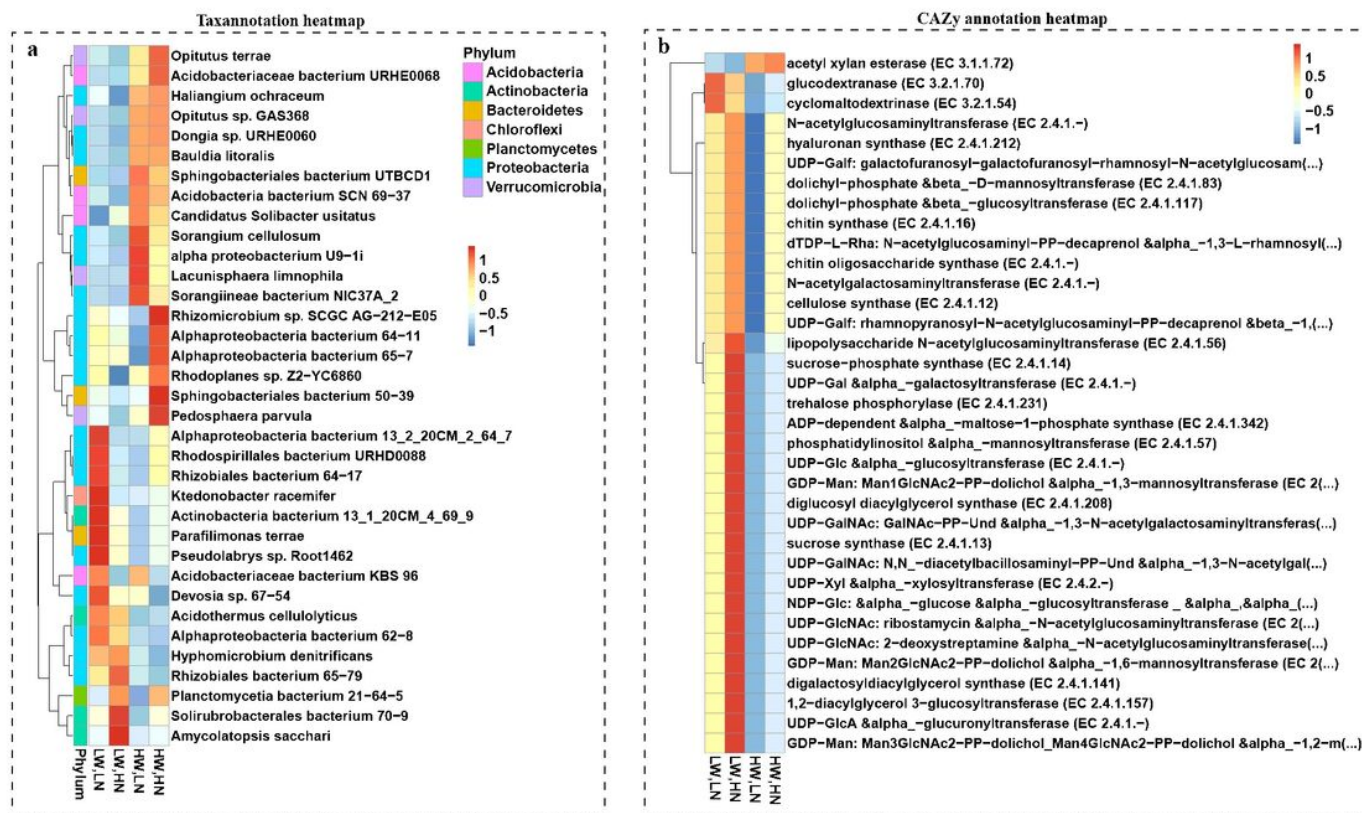


Figure 5

The heatmap of Taxannotation and CAZy (Carbohydrate-Active enZymes) annotation. Red color represents high expression level and blue color represents low expression level. LW,LN: low water and low nutrient; LW,HN: low water and high nutrient; HW,LN: high water and low nutrient; HW,HN: high water and high nutrient.

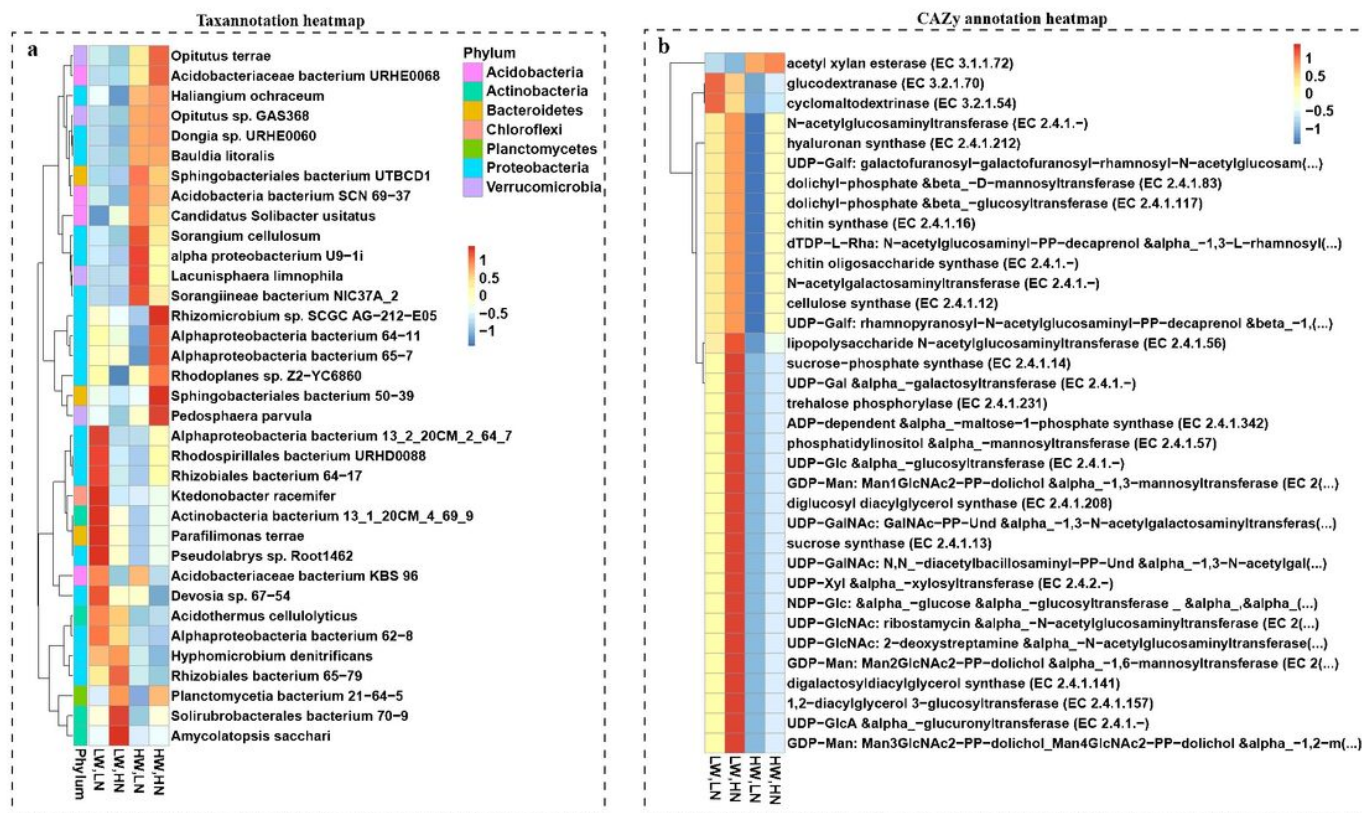


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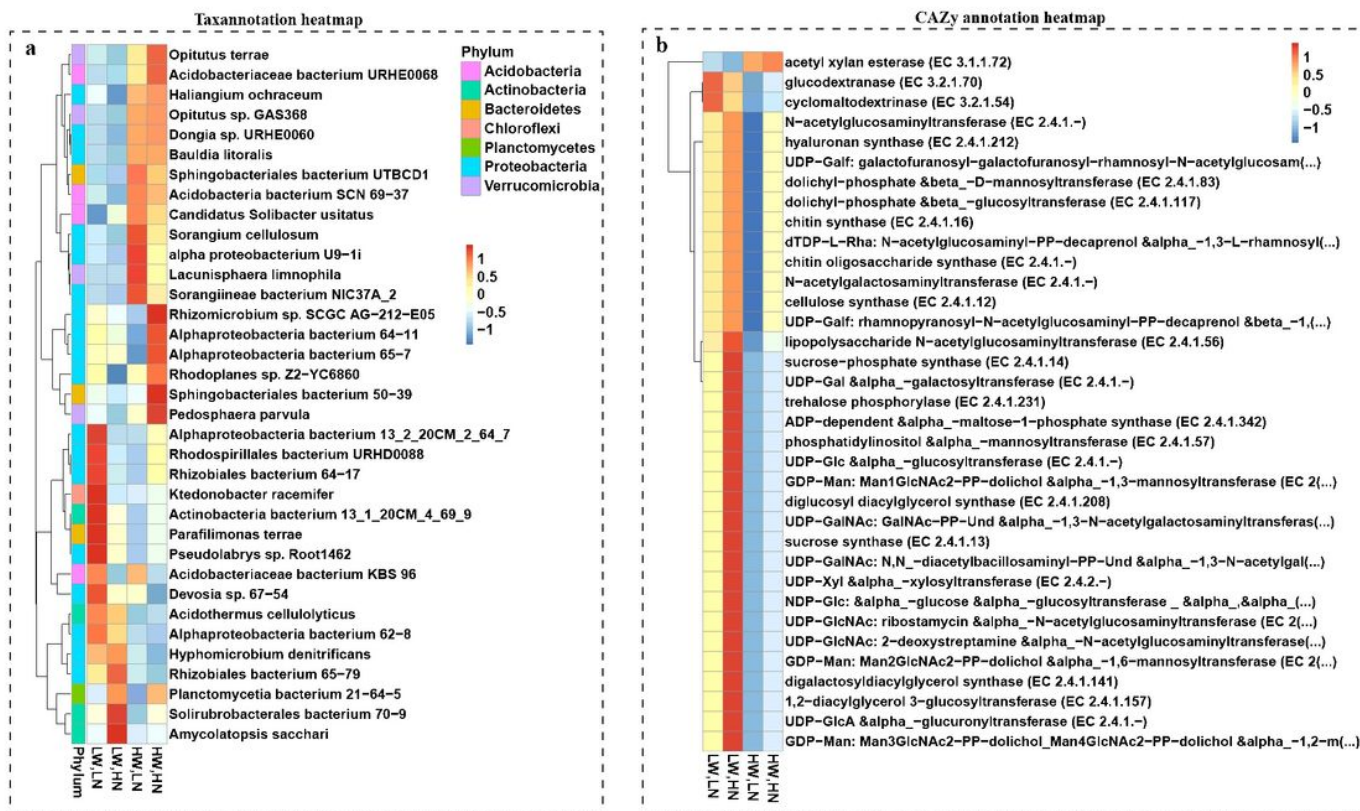


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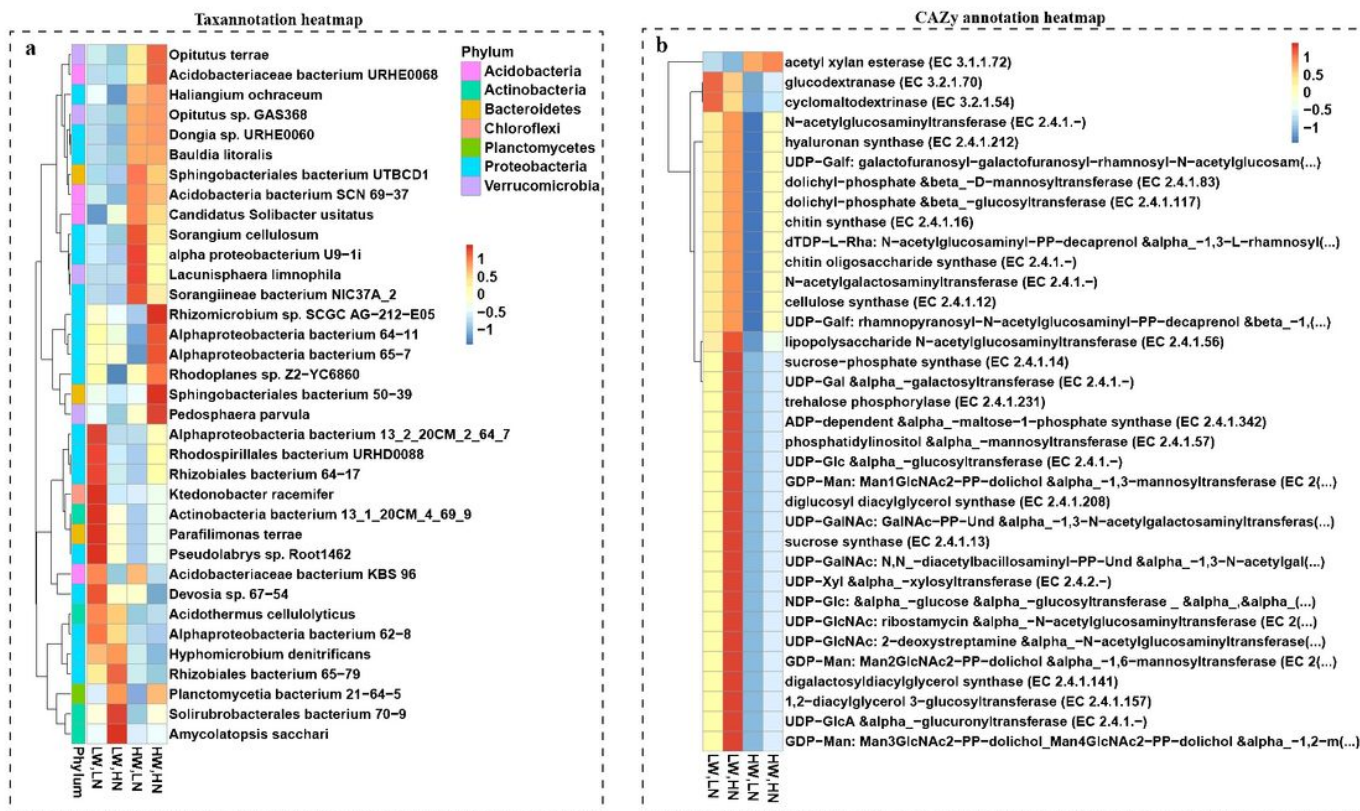


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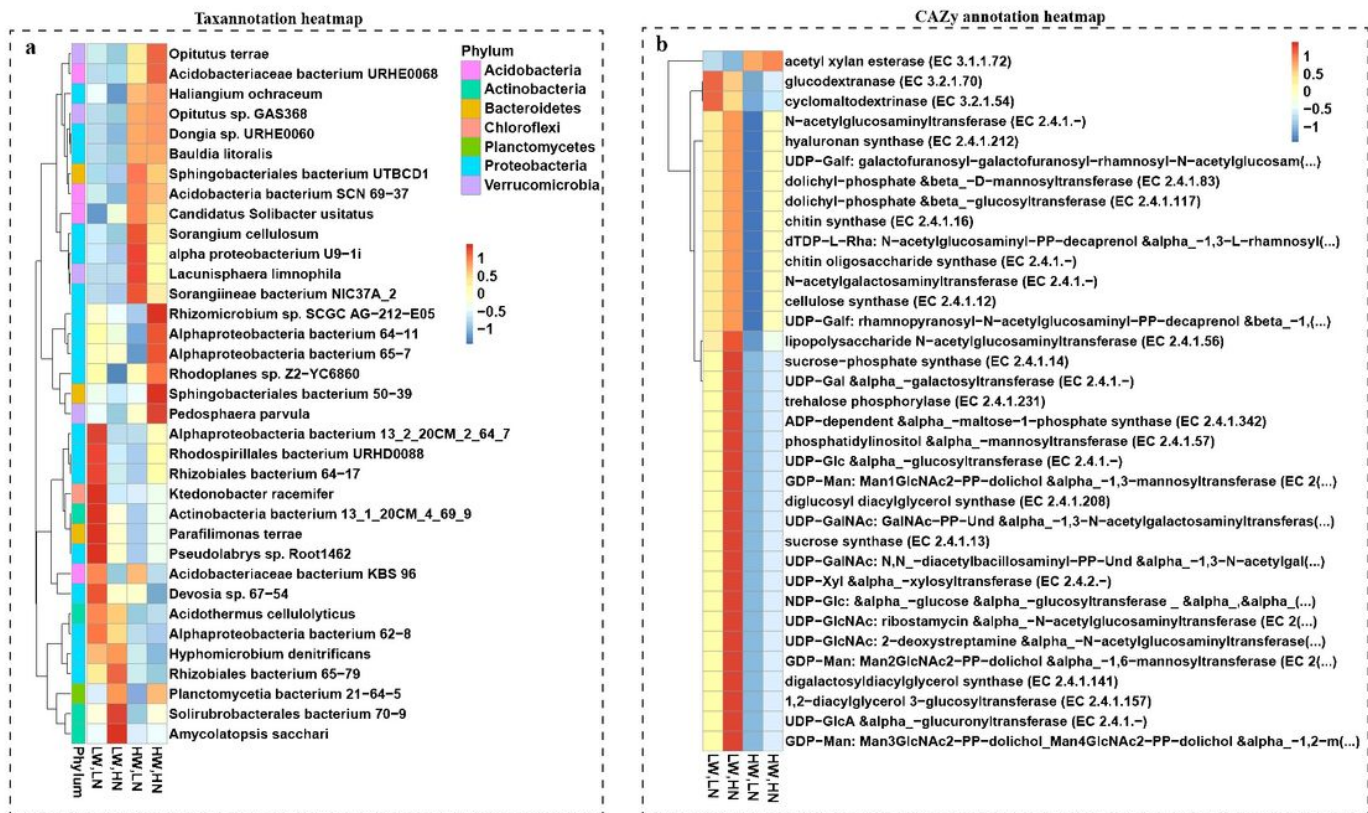


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