

Gut yeast diversity of *Helicoverpa armigera* under different dietary conditions

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Abstract

Intestinal endophytic fungi have played an important role in the nutrition, detoxification and development of host insects during natural co-evolution. Insects also provide suitable living environment for endophytic fungi. Insect endophytic yeast is also one of the important flora. Currently, it is little known about intestinal endophytic yeast of host feeding on different dietary conditions. *Helicoverpa armigera* is an important pest of economic crop in Xinjiang of China. However, the composition and function of intestinal yeast community in *H.armigera* in Xinjiang remains unclear. In this paper, the structural composition of yeast microorganisms in the larvae of *H.armigera* was explored under different environments feeding on apple, pear, tomato, artificial diet(laboratory feeding), urtica fissa, sunflower, and zinnia (wild environment) by high-throughput sequencing. Results showed that a total of 43 yeast OUT readings were obtained, including 33 yeast genera and 42 yeast species. The yeast genera with a total content of more than 5% were *Hanseniaspora* (36.27%), *Moesziomyces* (21.47%), *Trichosporon*(16.20%), *Wickerhamomyces* (12.96%) and *Pichia* (6.38%). *Hanseniaspora* was predominant when fed indoors with fruits, whereas *Moesziomyces* was only detected in the wild group (urtica fissa, sunflower, zinnia) and the artificial diet group. After the larvae were transferred from artificial diet to apple, pear and tomato, the structure of intestinal yeast community changed, mainly reflected in the increase of relative abundance of *Hanseniaspora* and the decrease of *Trichosporon*. At the same time, the results of a diversity index (Chao1) indicated that the intestinal yeast microbial diversity of *H.armigera* fed on wild plants was higher than that of indoor artificial feeding, and the highest was group Z (17.17 ± 4.37). PCoA and PERMANOVA analysis concluded that there were significant differences in the gut yeast structure of larvae of *H.armigera* on different diets which is likely to relate to host adaptation.

1. Introduction

The intestines of all metazoan harbor contain a abundant number of commensal microorganisms(Shin et al. 2011a, b).In the long-term co-evolution, insects provide a stable living environment and necessary nutrients for gut microorganisms, and gut microorganisms are also conducive to the survival of insects, which have an significant impact on the nutritional metabolism(Gong et al. 2020), growth and development(Lauzon, Sjogren, and Prokopy 2000, Yang et al. 2020), reproductive behavior(Yong et al. 2017), immune disease resistance(Johnston and Rolff 2015, Su et al. 2019) and other life processes of insects, forming a mutually beneficial symbiotic relationship(Dillon, R., and V. 2004, Shapira and Michael. 2016). It has been widely demonstrated that gut microorganisms can degrade exogenous toxins(Abdelgaffar 2019, Yang et al. 2020) and affect the transmission efficiency of vector insects(Fukatsu and Hosokawa 2002). On the other hand, it has been confirmed that diets with different nutritional differences can affect the longevity of insects(Edgar et al. 2011), and diet is one of the most prominent factors in the formation of intestinal bacterial communities(Harris, Roode, and Gerardo 2019, Moran, Ochman, and Hammer 2019). In the gut, microbial community tends to be diversified and stable(Angela, E., and Douglas 2015), and stable intestinal bacteria is conducive to maintaining the growth and development of insects(Colman, Toolson, and Takacs-Vesbach 2012). In order to promote the

understanding of the interaction between insect hosts and microorganisms, more and more people pay attention to the relationship between insect gut microbiota community structure and insect hosts (Angela, E., and Douglas 2015, Xu 2019).

Helicoverpa armigera is a worldwide pest with more than 200 host plants including cotton, tomato, pepper, potato and other crops and wild plants (Gu et al. 2022, Zheng et al. 2022). Because of its wide distribution, omnivorous, fast reproduction and strong adaptability (Fitt and P 1989), it has caused huge economic losses to global agriculture (Gujar et al. 2021). *Helicoverpa armigera* is widely distributed in China's cotton and fruit planting areas. It mainly eats the leaves of crops directly with the chewing mouthparts of larvae, or eats the fruits by drilling (Tsafack 2013), which poses a serious threat to crop yield and quality. Traditional insecticides are ineffective, and widespread use may lead to problems such as insecticide resistance, environmental pollution and human health. *Bacillus thuringiensis* (Bt), a biological agent commonly used in agriculture, has a certain effect on the prevention and control of agricultural and forestry pests. However, the diversity of gut microbiota in larvae makes it different in sensitivity to *Bacillus thuringiensis* (Downes et al. 2017, Wang et al. 2019). The reported gut microbiota of *Spodoptera littoralis* and *Manduca sexta* increased the susceptibility of larvae to *Bacillus thuringiensis* and enhanced the insecticidal activity (Broderick, Raffa, and Handelsman 2006, Caccia et al. 2016, Jonathan et al. 2009). The gut bacteria in *Galleria mellonella* and *Homona magnanima* enhanced the tolerance of larvae to *Bacillus thuringiensis* (Dubovskiy et al. 2016, Takatsuka and Kunimi 2000). In addition, larvae feeding on different host plants can affect the resistance of larvae to insect pathogens. Therefore, gut microbiome is the linchpin of the survival mechanism of insects on different hosts (Yuan et al. 2021).

Food can significantly change the composition of host microorganisms (Chen et al. 2018). It has been reported that wood-eating insects have more complex gut microbial communities than sap-eating insects (Akiko et al. 2015). Studying the changes of intestinal microflora of wood beetle moth under natural conditions and artificial feeding conditions, the results showed that the intestinal microbial diversity of wood beetle moth fed with different diets was significantly different (Ma et al. 2021). Yang et al. found that the intestinal microbial diversity of *Pxylostella* was significantly reduced after 17 generations of transfer from host radish to pea (Yang et al. 2020). But as far as we know, when *H. armigera* feeds on different fruits and flowers, there are few reports on the structure of gut microbiota, and even fewer studies on the structure of gut yeast community.

To explore the impact of diets on the gut yeast communities of larvae of *Helicoverpa armigera*, 26S rDNA sequencing technology was used to study the structure of intestinal yeasts of *Helicoverpa armigera* fed on artificial diet, apple, tomato, pear (laboratory feeding), sunflower, urtica fissa and Zinnia (wild environment). The results revealed the changes of intestinal yeast community in different hosts, and speculated the potential relationship between *H. armigera* and intestinal yeast community, which provided a new idea for the prevention and control of *H. armigera* in agriculture.

2. Results

2.1 Analysis of 26S rDNA high-throughput sequencing

Illumina HiSeq technology was used to sequence 21 samples from 7 treatments. After splicing and filtering, a total of 1296261 clean tags were generated. Each sample generated an average of about 62,727 clean labels. Based on 97% sequence similarity clustering analysis, 224 OTUs were obtained, including 22 phyla, 47 classes, 92 orders, 130 families, 164 genera and 191 species. The total reads and OTU numbers of yeast were 40703 and 43 respectively, including 2 phyla, 9 classes, 12 orders, 20 families, 33 genera and 42 species. The sample Shannon index dilution curve (Fig. 1) shows that the sequencing amount is sufficient, the sequencing depth is saturated, and increasing the sample size will not produce more OTUs.

2.2. Comparison of intestinal gut yeast groups of *Helicoverpa armigera* under different hosts.

The α diversity index (Table 1) showed that there were differences in the community structure of intestinal yeasts in *H. armigera* fed on different diets. The abundance of yeasts in the intestinal tract of *H. armigera* fed on wild plants (UF, S, Z) was higher than that of laboratory artificial fruit feeding group (A, T, P). Especially, the abundance of yeasts in the intestinal tract of wild *H. armigera* fed on *Zinnia elegans* (Z) was significantly higher than that of laboratory artificial feeding group (CK, A, T, P), and the Chao 1 index was 17.17 ± 4.37 a. The richness of UF, S and Z groups was similar, and there was no significant difference between CK, A, T and P groups. According to the difference analysis between groups, the Shannon index of S group was the highest, and the yeast diversity was significantly higher than that of A and P. When the cotton bollworm was transferred from artificial feed to apple, tomato and pear, the diversity of intestinal yeast population did not change significantly.

Table 1
Alpha diversity indices of yeast in insect gut from different samples

Sample ID	Chao1	ACE	Shannon	Simpson
UF	12.37 ± 3.90ab	15.46 ± 4.22ab	1.27 ± 1.10ab	0.40 ± 0.51ab
S	12.33 ± 2.89ab	12.83 ± 1.63abc	1.95 ± 0.10a	0.15 ± 0.06b
Z	17.17 ± 4.37a	18.62 ± 3.67a	0.66 ± 0.33 b	0.72 ± 0.13a
CK	7.83 ± 3.33b	8.45 ± 3.26bc	1.21 ± 0.22ab	0.40 ± 0.11ab
A	10.42 ± 1.51b	6.69 ± 5.89c	0.69 ± 0.27b	0.65 ± 0.16a
T	9.00 ± 1.73b	16.40 ± 7.82ab	1.13 ± 0.14ab	0.40 ± 0.03ab
P	8.33 ± 0.29b	14.23 ± 5.52abc	0.41 ± 0.16b	0.80 ± 0.13a
Note: Samples abbreviations are as in Table 1. Each sample had three replicates. Sobs index was the observed species richness, Chao1 and ACE indices were used to evaluate species richness, Shannon and Simpson indices were used to evaluate species diversity. Larger Simpson index values indicate lower species diversity. The values of mean ± SE (standard error) of three samples are shown in the table. The different lowercase letters are significantly difference within groups, the different capital letters are significantly difference among groups. (Kruskal-Wallis test, P < 0.05).				

After read quality filtering, the representative sequences of OTUs were compared with the NCBI reference database to obtain the species classification information corresponding to each OTU, and then the composition of each sample was counted at the genus and species levels. At the genus level (Fig. 2a), *Hanseniaspora* (36.27%), *Moesziomyces* (21.47%), *Trichosporon* (16.20%), *Wickerhamomyces* (12.96%) and *Pichia* (6.38%) had the total relative abundance greater than 5%.

Hanseniaspora is an absolutely dominant genus, and its relative abundance in artificial feeding samples is generally higher than that in wild sampling groups. The relative abundance of apple feeding group (A) is the highest, which is 77.05 ± 14.18%. The other three groups are : artificial feed feeding group (CK) is 3.97 ± 6.34%, tomato feeding group (T) is 44.54 ± 11.64%, pear feeding group (P) is 68.78 ± 41.66%, and this genus is not detected in QMM group. On the contrary, the relative abundance of *Moesziomyces* in the artificial feeding samples was lower than that in the wild sampling group, and the highest Z group was 90.45 ± 10.94 a, which was not detected in the artificial feeding group (A, T, P). The relative abundance of *Trichosporon* was higher in the apple feeding group (A) and the feed feeding group (CK). The relative abundance of *Wickerhamomyces* was only more than 5% in the UF group, which was 40.04 ± 51.68 a, while the relative abundance of *Pichia* was not significantly different in the 7 groups. Obviously, the relative abundance of *Hanseniaspora* was significantly increased in other groups and the relative abundance of *Trichosporon* was decreased after feeding *H.armigera* from feed to different fruits (Fig. 2b c, Table 2).

Table 2
The relative abundance of the top five genus

Sample ID	<i>Hanseniaspora</i>	<i>Moesziomyces</i>	<i>Trichosporon</i>	<i>Wickerhamomyces</i>	<i>Pichia</i>
UF	0.00 ± 0.00 c	13.56 ± 17.50 b	10.11 ± 17.41 c	40.04 ± 51.68 a	3.48 ± 6.01 c
S	2.34 ± 4.05 c	14.64 ± 10.94 b	34.28 ± 6.30 b	3.03 ± 5.25 b	7.66 ± 7.05 b
Z	0.04 ± 0.03 c	90.45 ± 10.94 a	1.51 ± 1.15 c	0.00 ± 0.00 b	0.01 ± 0.01 c
CK	3.97 ± 6.34 c	1.57 ± 2.75 b	56.93 ± 12.62 a	0.00 ± 0.00 b	6.27 ± 10.85 b
A	77.05 ± 14.18 a	0.00 ± 0.00 b	18.32 ± 11.24 bc	0.00 ± 0.00 b	0.27 ± 0.25 c
T	44.54 ± 11.64 b	0.00 ± 0.00 b	3.37 ± 1.67 c	0.57 ± 0.76 b	2.35 ± 2.35 c
P	68.78 ± 41.66 ab	0.00 ± 0.00 b	0.42 ± 0.23 c	0.00 ± 0.00 b	28.92 ± 43.32 a
Note: The values of mean ± SE (standard error) of three samples are shown in the table. The different lowercase letters are significantly difference within groups, the different capital letters are significantly difference among groups. (Kruskal-Wallis test, P < 0.05).					

In order to count the number of species in multiple samples and the information of common and unique species, we drew a Venn diagram at the species level (Fig. 3). A total of 52 species were detected in all intestinal samples. Further analysis showed that there were 12 yeast species in wild samples and laboratory artificial samples(Fig. 3a). We also found that each sample has its own unique yeast species. At the species level, the highest detected 22 species (group Z) and the lowest 10 species (T group) (Fig. 3b). In all samples, *Trichosporon* sp.and *Cutaneotrichosporon curvatum* were common species, and three unique species-*Sympodiomyces* sp., *Pichia occidentalis*, *Clavispora lusitaniae* and *Pichia terricola* appeared in sample S(Table 3).

Table 3
Exclusive and shared species in all samples

Sample	Only species	All shared species
QMM	<i>Metschnikowia pulcherrima</i>	<i>Trichosporon</i> sp. <i>Cutaneotrichosporon curvatum</i>
XM	<i>Sympodiomyces</i> sp.	
	<i>Pichia occidentalis</i>	
	<i>Clavispora lusitaniae</i>	
	<i>Pichia terricola</i>	
C	<i>Papiliotrema laurentii</i>	
	<i>Sporobolomyces roseus</i>	
	<i>Papiliotrema</i> sp.	
SL	<i>Malassezia</i> sp.	
	<i>Exophiala lecanii-corni</i>	
PG	<i>Kondoa gutianensis</i>	
	<i>Candida saitoana</i>	
	<i>Kazachstania slooffiae</i>	
XHS	<i>Wickerhamomyces pijperi</i>	
	<i>Kurtzmaniella natalensis</i>	
L	no	

The results of species difference analysis based on the phylum level showed that the yeast species in the intestinal samples of wild *H.armigera* were mainly concentrated in *Basidiomycetes*, while the laboratory feeding samples were mainly *Ascomycota* (Fig. 4a). At the genus level (Fig. 4b), there were significant differences in 6 genera ($P < 0.05$) and extremely significant differences in 5 genera ($P < 0.01$) among the 7 groups. *Moesziomyces* and *Trichosporon* mainly existed in UF, S and Z. *Hanseniaspora* and *Trichosporon* were mainly detected in CK, A, T and P.

In order to reveal the dynamic changes of intestinal microflora of *H.armigera* feeding on different hosts, we selected 10 genera with the highest relative abundance to draw the relative abundance cluster heat map. Clustering is based on the similarity of species abundance, horizontal clustering is sample information, and vertical clustering is species information. The wild plants *Urtica fissa* (UF), *Zinnia elegans* (Z) and *Helianthus annuus* (S) are eaten on different branches. The composition of gut yeast community of *H. armigera* feeding on artificial diet (CK) changed at genus level after transferred to apple (A), pear (P) and tomato (T), and clustered on different branches. The composition of gut yeast

community of *H. armigera* feeding on pear (P) and tomato (T) was the most similar. *Moesziomyces* is a major component of the gut microbiome of *H. armigera* feeding on *Zinnia elegans*, and the relative abundance of *Wickerhamomyces* in the gut of *H. armigera* feeding on *Urtica* is much higher than in other groups, as are *Hanseniaspora* and *Trichosporon* in the A group (Fig. 5).

Sorted at the OTU level by PCoA to compare community composition similarities between samples. The abscissa and ordinate respectively explain the two eigenvalues that contribute the most to the difference between the samples, and the influence degree is 20.89% and 15.49% respectively (Fig. 6). PCoA1 has relatively small eigenvalues and captures less than 50% of the changes in the input data, so it is not considered to be a very successful PCoA. However, the results of Adonis analysis (Fig. 7) showed that the R^2 value ($R^2 = 0.6281$, $P = 0.001$) was greater than 0 and tended to 1, indicating that the difference between the sample groups was greater than that within the sample group. Among the seven insect gut samples, most of the samples in each group clustered together, the three replicates of each sample had good reproducibility, and the degree of separation between each sample group was better, with an AP-value < 0.05 ($P = 0.001$), indicating that there were significant differences in community composition between groups.

3. Discussion

Helicoverpa armigera is an omnivorous and highly migratory agricultural pest. The hatched larvae feed on tender leaves, flowers or fruits of plants (Luo et al. 1999). The growth, development and survival rate of *H. armigera* are affected by different host plants (Dongsoon and Joonho 2002). Insect gut microbiota ecosystem inhabits a large number of microorganisms (Flory, Clay, and Emery 2015), and insects can use a variety of evolutionary mechanisms with symbiotic bacteria to assist them in feeding on plants (Frago, Dicke, and Godfray 2012). However, the dynamic changes of intestinal yeast populations in *H. armigera* feeding on different hosts are not completely clear. Therefore, we first revealed the differences in intestinal yeast diversity and community composition of *H. armigera* larvae fed on wild plants and artificial laboratory fruits by high-throughput sequencing. According to our experimental results, the diversity and relative abundance of the intestinal yeast community of *H. armigera* are affected by the host diet. There is a complex yeast symbiotic community in the intestinal tract of *H. armigera* larvae, which provides a theoretical basis for explaining the adaptation mechanism of *H. armigera* and its host plants.

Studies have shown that the intestinal symbiotic bacterial diversity of cotton boll larvae fed on different geographical regions and different cotton tissues will be affected (Zhao 2021). In this experiment, it was found that there were differences in the structure of yeast population after the cotton boll larvae were transferred from artificial feed to pear, apple and tomato. The diversity of yeast in the intestinal tract of cotton boll larvae fed on wild plants (UF, S, Z) was higher than that of the laboratory artificial fruit (CK, A, T, P) feeding group, and the diversity of yeast in the intestinal tract of cotton boll larvae fed on *Zinnia elegans* was significantly higher than that of the laboratory feeding group. Some researchers have reached a similar conclusion that the diversity of symbiotic bacteria in cotton boll larvae fed on cotton is higher than that of cotton boll larvae fed on feed (Zhao 2021). It was also confirmed that the diversity of

intestinal bacteria decreased after *Plutella xylostella* was transferred from radish to pea, and the structure of intestinal bacteria varied with feeding (Yang et al. 2020).

At the phylum level, the intestinal yeast population of cotton boll larvae feeding on plants was mainly concentrated in *Basidiomycetes*. On the contrary, the intestinal yeast population of cotton boll larvae feeding on fruits in the laboratory was dominated by *Ascomycota*. At the genus level, *Hanseniaspora* was mainly detected in fruit-fed *H.armigera*. This may be due to the fact that *Hanseniaspora* is the dominant yeast genus in the early stage of natural fermentation, which is widely found on the epidermis of mature fruits and can secrete a variety of hydrolases. Some strains of this genus have the characteristics of low ethanol production, which is beneficial to help insects digest the pulp and do not cause damage to host insects (Capece et al. 2005, Hong and Park 2013, Viana et al. 2009). *Moesziomyces* spp. belongs to *Basidiomycota* yeast, which was originally isolated from the sediments of Lake Wanda, Antarctica (Li et al. 2019) and are mostly isolated from plant surfaces, providing a source of natural products resistant to plant powdery mildew (Liu et al. 2019), which is consistent with this study. The relative abundance of *Trichosporon* was higher in the artificial diet (CK), similar to that of *Wickerhamomyces* in the wild nettle (UF) group. These results strongly suggest that the intestinal endophytic yeast populations of *H. armigera* are related to the yeast populations on the host, and there may be some similarities. Insect gut microbial communities are affected by host diet, and the dominant microbial lineages are different (Ayayee, Larsen, and Sabree 2016, Bing et al. 2018, Liu et al. 2020, Vilanova et al. 2016).

Trichosporon spp. can be isolated from a variety of habitats, including soil, wastewater, sediment, wood pulp and sludge, etc. (Kurtzman 1998). It and use their cells or enzyme systems to metabolize pollutants and exogenous substances during bioremediation when use uric acid, ethylamine, aniline and aromatic compounds as the sole energy source (Bergauer et al. 2005, Godjevargova and Gabrovska 2003). *Cutaneotrichosporon curvatum* can also use unusual carbon source to produce oil and has the ability to accumulate lipid biological functions can undoubtedly provide the necessary nutrients for the growth of cotton boll larvae or help the host detoxify (Péter et al. 2019). Among the unique species of each sample, *Metschnikowia pulcherrima* detected in the wild group can produce a variety of acidic proteases and microbial lipids (Li et al. 2021); group Z *Papiliotrema laurentii* is an oleaginous yeast with a diversity of metabolic carbon sources that can produce enzymes and high concentrations of lipids (de Almeida et al. 2022); similarly, *Sporobolomyces roseus* can produce zeatin (Streletskii et al. 2019) and degrade amino acids (Moore, Rao, and Towers 1968). In addition, *Papiliotrema* has been reported to be resistant to heavy metals (Nguyen, Nguyen, and Truong 2020). In contrast, the unique species detected in the indoor artificial feeding group were simple and less reported. This difference may be due to the complex living environment of wild *H.armigera*, such as sunshine, temperature, water and food. In order to adapt to the changing natural environment, more symbiotic bacteria with special functions are needed to help them resist external adverse factors. In addition, some human pathogenic yeasts-*Clavispora lusitaniae*, *Malassezia* sp., *Exophiala lecaniicorni* appeared in field samples and feed groups.

PCoA analysis showed that there were differences in the composition of intestinal yeast populations. We also found that there was no significant difference in the structure of intestinal yeast microflora between

the tomato-fed group (T) and the pear-fed group (P) (Permonova : $R^2 = 0.275$, $P = 0.3$). Here is a bold conjecture to explain this point. Tomatoes and pears have some common similar nutrients, which may be amino acids or acids that provide the growth needs of cotton boll larvae (Shao et al. 2011). Plant secondary metabolites and host nutritional requirements affect the composition of gut microbiota (Blanco et al. 2007, Muhammad et al. 2017, Myers, Hull, and Krawczyk 2006). The host of insects is affected by many factors, such as environment, dietary maturity, host volatiles, etc., and many influencing factors can indirectly affect the composition of insect intestinal symbiotic microbial community. There is a close relationship between the three.

In conclusion, our results confirmed that the intestinal yeast community structure of cotton boll larvae was affected by host feeding. The diversity of *H. armigera* fed indoors was lower than that fed in the field. Under different host feeding, the dominant yeast species were different. *Hanseniaspora* and *Trichosporon* were fed indoors, and *Moesziomyces* were fed in the wild group. When the cotton boll larvae were transferred from artificial feed to apple, pear and tomato, the abundance of each yeast population changed, increased or decreased. These changes may play a role in the mutual adaptation between microorganisms and hosts. Of course, our research has many limitations. High-throughput sequencing technology can only reflect the changes of microbial community structure macroscopically. Future research to further clarify the mechanism of this change and the important role of intestinal flora in the host, should be through multi-omics analysis to find new ways to control cotton boll pests.

4. Materials and Methods

4.1 Insect sample acquisition

Laboratory feeding *Helicoverpa armigera* was collected from Shehezi University, which was kept in the artificial diet of $26 \pm 0.5^\circ\text{C}$ $70 \pm 10\%$ relative humidity Rh and 15H 9H photoperiod L:D for more than 3 generations. The fourth generation of newly hatched eggs were fed with artificial diet CK , apple A , pear P and tomato T to the fourth instar. All host plants were collected from Botanical Garden of Shihezi University in 2021. And wild third instar larvae were collected from hanada in Shihezi City($N:44^\circ15'23.14''$ $E 86^\circ03'9.68''$).

4.2 DNA Extraction

30 healthy individuals were selected and disinfected with 75% ethanol for 60 s, then disinfected with 0.5% sodium hypochlorite for 30 s, and finally washed with sterile water for three times for 60 s. The midgut tissue dissected with sterile clamp and washed again in sterile phosphate buffer saline(PBS). 30 midguts were collected from each sample using sterile centrifuge tube. Three replicates per treatments. FastDNA Spin Kit for Soil(MP Biomedicals, UAS)was used to extract the total DNA of samples.

4.3 PCR Amplification and High-Throughput Sequencing

Yeast 26S rDNA was amplified using a pair of specific primers NL1F(forward primer) (5' - GCATATCAATAAGCGGAGGAAAAG-3') and NL2 R(reverse primer)(5' -CTTGTTCGCTATCGGTCTC-3') (Keidser et al. 2012). The target DNA bands were amplified on a PCR thermocycler(ABI, California, CA, United States)with an denaturation at 98 ° C for 5 min, followed by 98 ° C cycle for 30 s, 52 ° C cycle annealing for 30 s, and finally extension at 72 ° C for 45 s. PCR products were analyzed using 1.8 % agarose gel electrophoresis and finally purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, USA). Quantitative TM-ST (Promega, USA)of the target fragment using QuantiFluor(Zhang et al. 2018). The Illumina MiSeq PE300 platform(Illumina, USA) was used to perform paired end sequencing (2 × 300) of qualified libraries according to the instructions of Meiji Biomedical Company(Shanghai, China).

4.4 Statistical and Bioinformatics Analysis

The software Trimmomatic (version 0.33, Golm, Germany)was used to filter the quality of the original sequence file, and then the software cutadapt (version 1.9.1, TU Dortmund, Germany)was used to identify and delete primer sequences with high quality readings without primer sequences(Bolger, Lohse, and Usadel 2014). Through FLASH (version 1.2.7, Baltimore, MD, USA)software, the high-quality readings of each sample are spliced by overlapping, and the resulting splicing sequence is a clean reading(Magoč and Salzberg 2011). We use UCHIME (version 4.2) to identify and delete chimera sequences and obtain the final valid data as a valid read. According to the sequence similarity, the effective OTUs were clustered at 97 % similarity level by software USEARCH (version 10.0)(Edgar et al. 2011). The classification of each D1 domain of the LSU rRNA sequence was analyzed by the ribosomal database project (RDP) classifier algorithm (version 2.2). The confidence threshold used in the NCBI database (National Biotechnology Information Center) was 0.7. Next, we plot sparse curves to observe the community abundance and sequencing data of each sample(Li et al. 2018). Principal coordinate analysis (PCoA) was performed based on Bray Curtis at the OTU level, and the ' vegan ' and ' ape ' packages in R (version 4.0.2) were used to analyze the similarity or difference of sample community composition(Schloss et al. 2009). Difference tests between groups in PCoA were analyzed using ANOSIM (Similarity Analysis) using the vegan package in R. Alpha diversity (CHA01 estimator, ACE index, Shannon diversity index and Simpson index) and yeast abundance were analyzed using SPSS 26.0 (IBM, NY, USA)(Zhu et al. 2021). All values are expressed as mean ± SE. When $p < 0.05$, the difference is considered significant, and when $p < 0.01$, the difference is considered extremely significant.

Declarations

Supplementary Materials: S1–S3: Supporting data sets.

Author Contributions: Conceptualization, M.Y. and Y.L.; investigation, M.Y. and Y.L.; methodology, Y.L.; software, Y.L.; writing—original draft preparation, M.Y.; writing— review and editing, Y.S.; visualization, J.J.; project administration, J.J. and Y.L. All authors have read and agreed to the published version of the manuscript.

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Figures

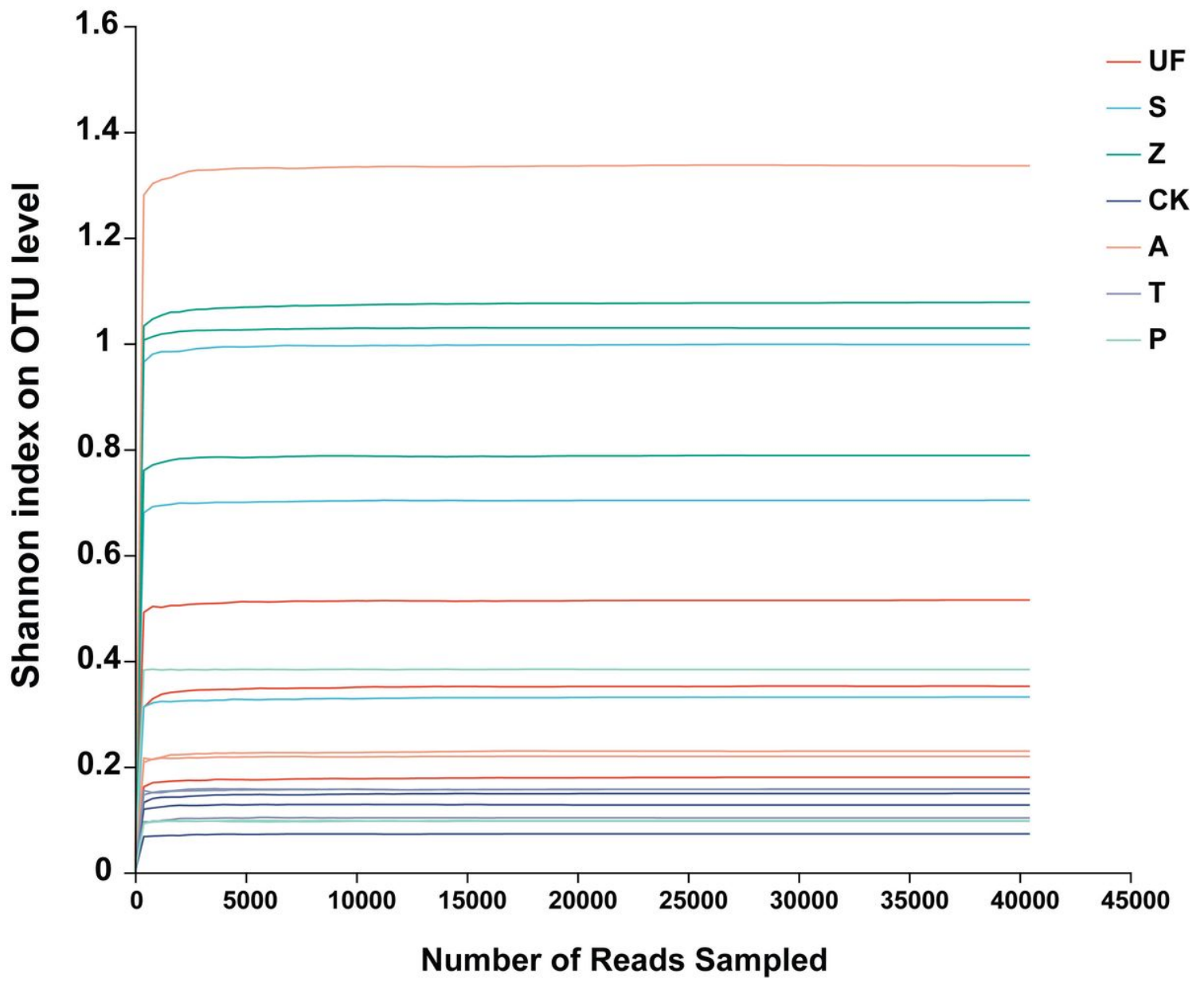


Figure 1

Rarefaction curves of insect gut from different samples. Rarefaction curves of OTUs were clustered for a dissimilarity threshold of 3%. Each sample had three replicates (Replicates are not specifically shown in the legend, but have been involved in the analysis). Here UF S Z CK A T and P represent the samples of *H. armigera* fed apple, pear, tomato, artificial diet, urtica fissa, sunflower, and zinnia. The following chart is the same .

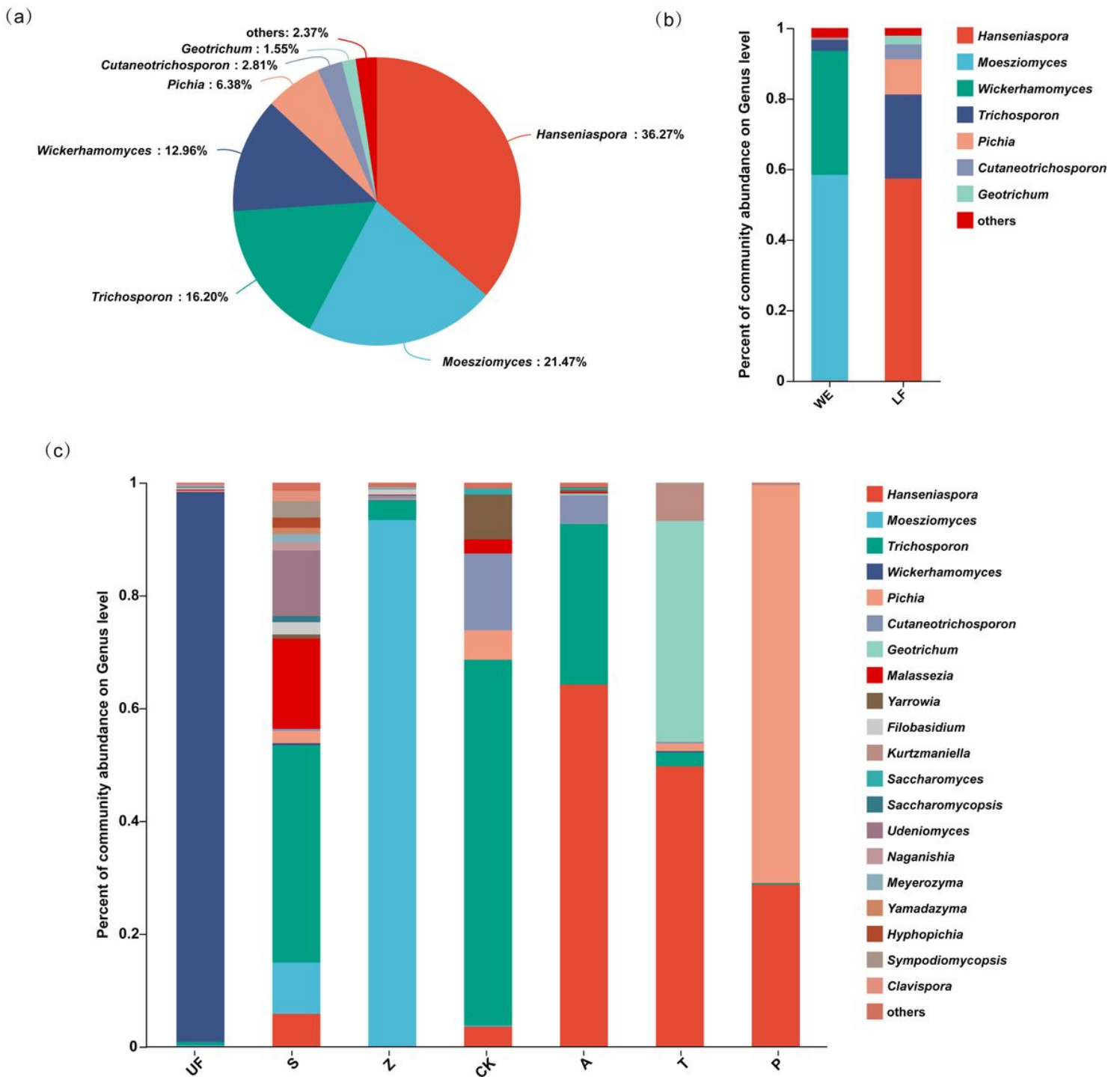


Figure 2

Each sample had three replicates. a is the yeast community analysis diagram of all samples at the genus level. b is the relative abundance of yeast in laboratory feeding samples LF includes CK A T and P samples and wild environmental samples WE includes UF S Z samples at the genus level. c is the relative abundance of yeast at the genus level for each sample.

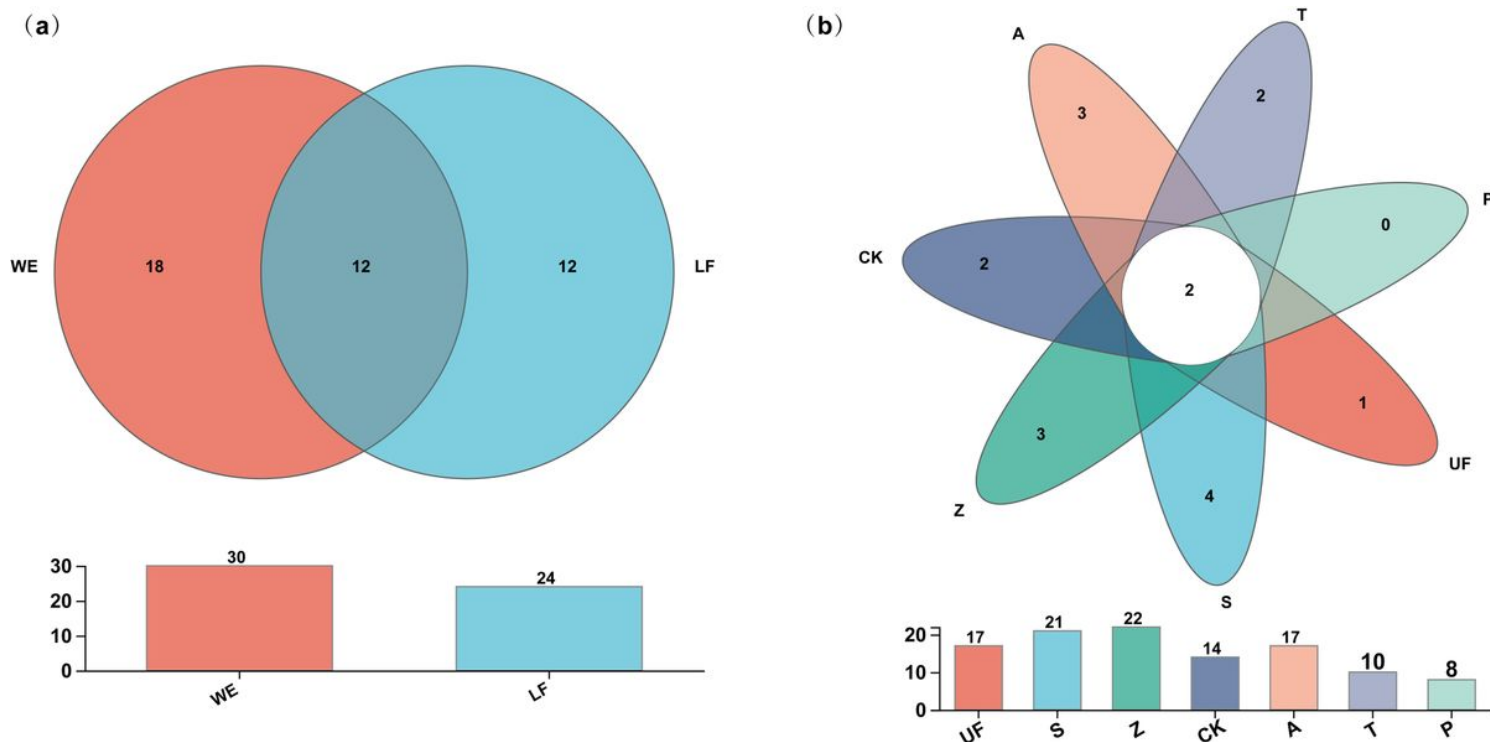


Figure 3

Venn diagram at the species level of samples. (a) represents laboratory feeding sample LF includes CK A T and P samples and wild environmental samples WE includes UF S Z samples. (b) represents each sample. Each circle with different colors in the diagram represents a group; middle core numbers represent the number of species common to all groups

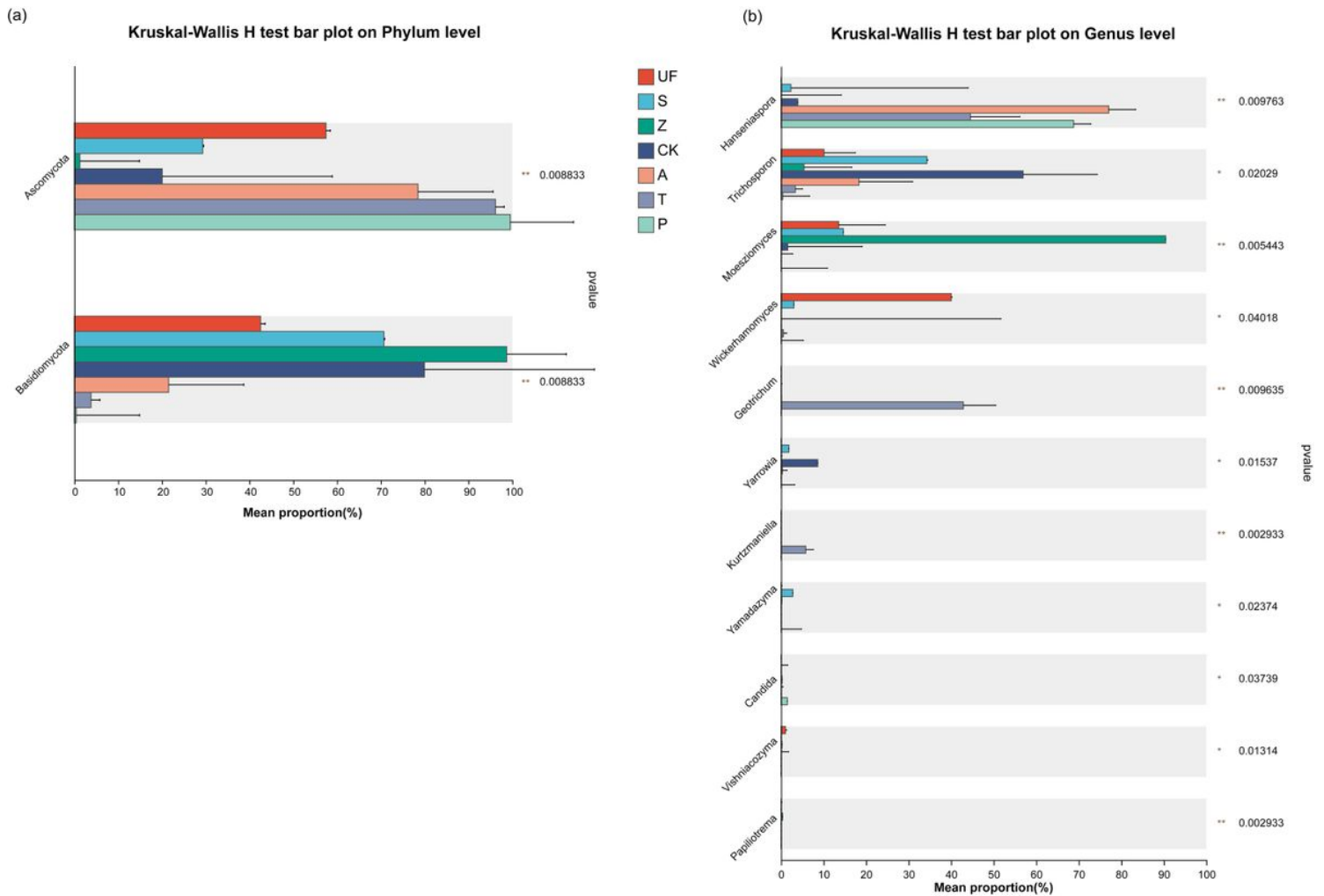


Figure 4

Species difference analysis of seven samples: (a) at the phylum level; (b) at the genus level. The y-axis represents the classification levels of species, and the x-axis represents the percentage of species average relative abundance in each sample group. Different colors represent different samples. The Kruskal-Wallis rank-sum test was used to show significant differences (*: $0.01 < P \leq 0.05$, **: $0.001 < P \leq 0.01$, ***: $P \leq 0.001$).

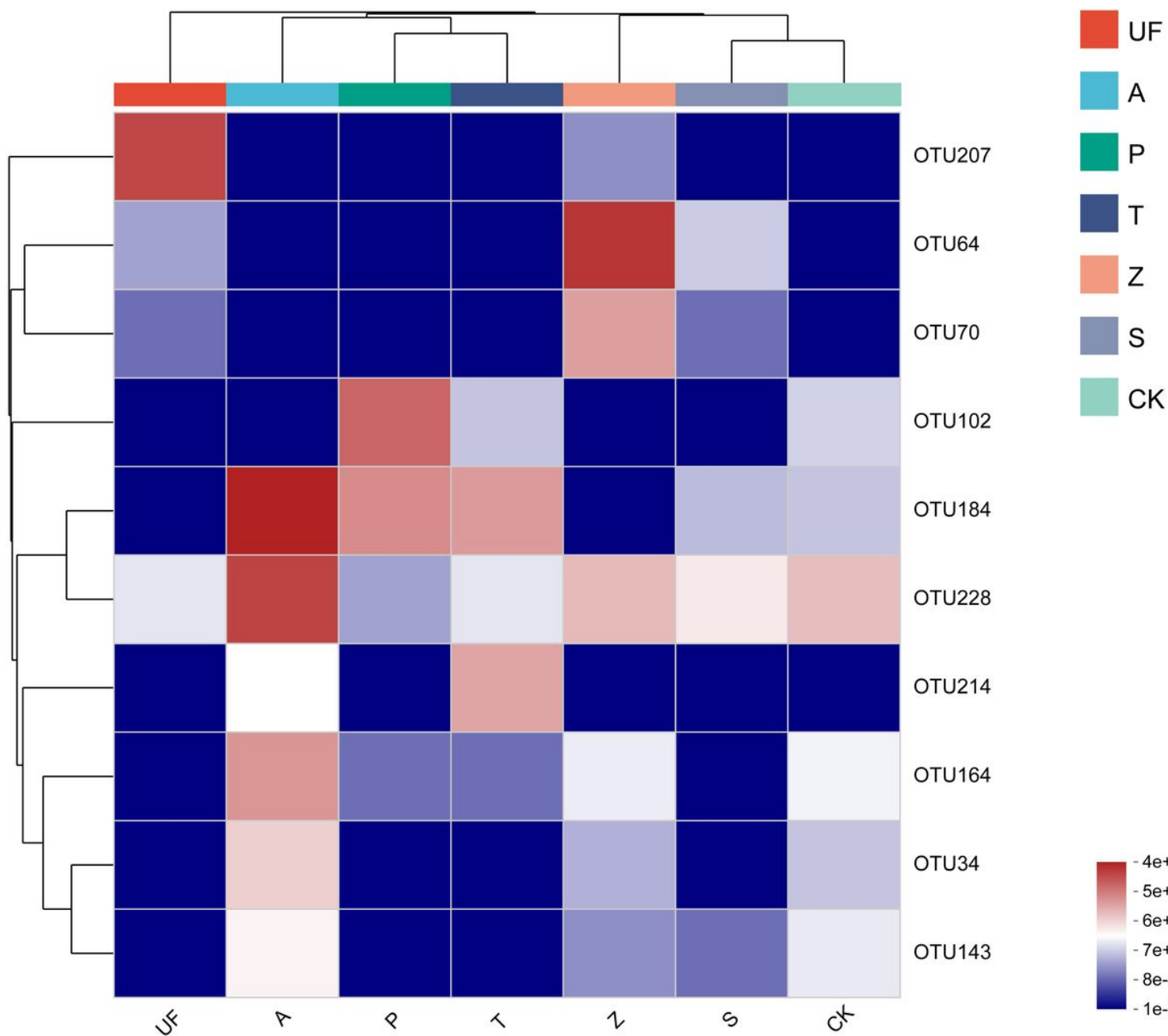


Figure 5

Cluster heat map of the 10 most abundant genera in the yeasts community. The columns represent the samples and the rows represent the yeasts OTUs assigned to the genus level. Dendrograms of hierarchical cluster analysis grouping genera and samples are shown on the left and at the top, respectively.

PCoA on OTU level

R=0.6281, P=0.001000

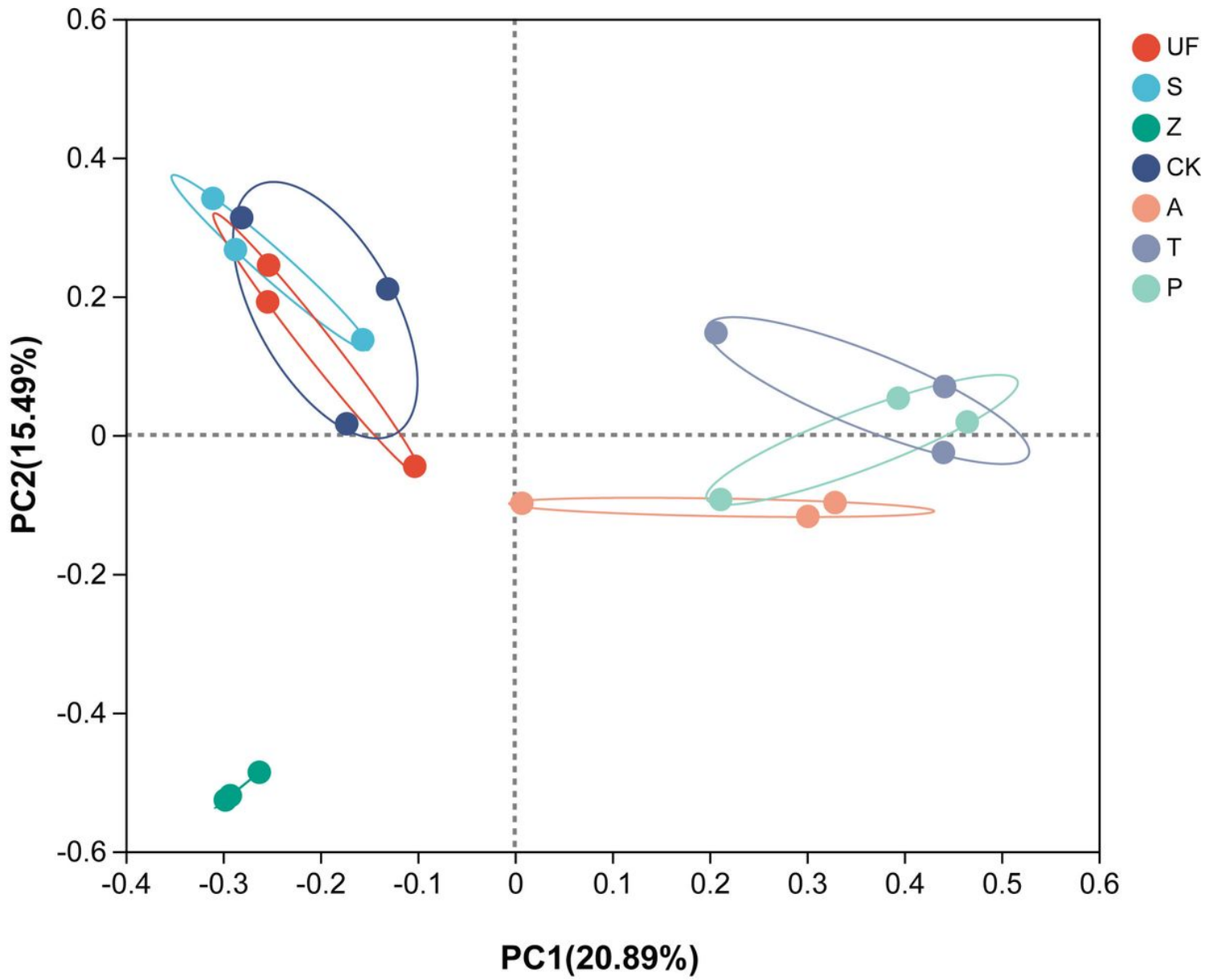


Figure 6

Principal Coordinates analysis (PCoA) based on Bray-Curtis distance method at the OTU level.

Distances box plot

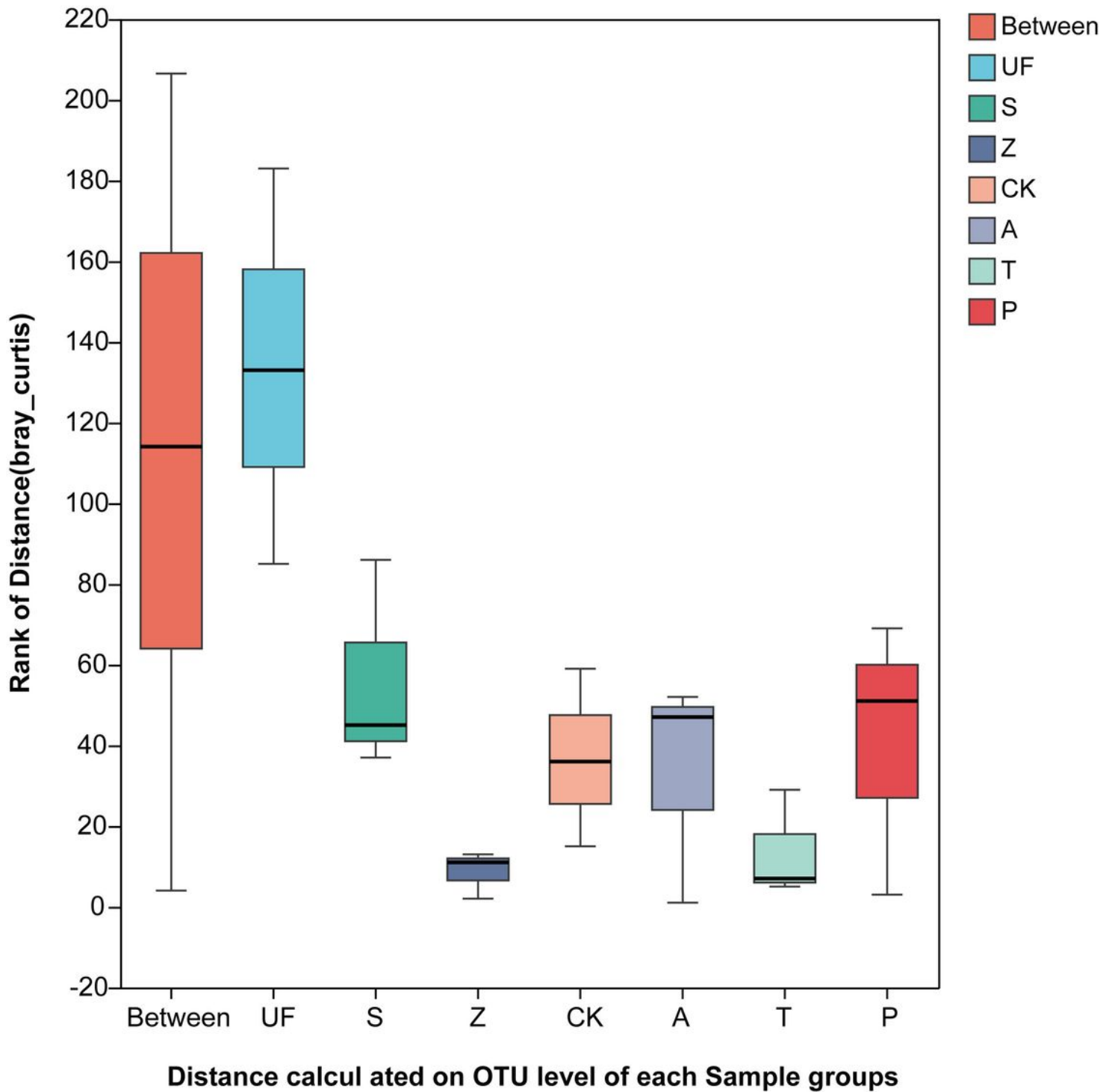


Figure 7

Adonis analysis account for the sample differences by different grouping factors. The “Between” boxes refer to differences between groups, while the others represent differences within their respective groups

Supplementary Files

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

- [S1.docx](#)
- [S2alphadiversity.csv](#)
- [S3OTUTable.csv](#)