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MICROBIAL COMMUNITY OF *FLAVERIA BIDENTIS* (L.) KUNTZE RHIZOSPHERE AND NON-RHIZOSPHERE SOILS IN THE YELLOW RIVER DELTA

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Abstract

Invasive plants have become an environmental issue of common global concern, posing a significant threat to environmental protection. As one of the invasive species, *Flaveria bidentis* (L.) Kuntze has seriously affected the ecological preservation of the invaded area. In recent years, most research on the invasion of *F. bidentis* to soil ecosystems has focused on soil nutrient content, bacterial and fungal community diversity. In comparison, the effect of the invasive plant on the Yellow River Delta (YRD) was still unknown. We obtained the soil microorganisms under *F. bidentis* invasion in the present study. At the phylum level, two groups including the rhizosphere soil bacteria samples of *F. bidentis* (HDJ) and the bulk soils samples of *F. bidentis* BSK groups were dominated with the Proteobacteria, Acidobacteriota, Gemmatimonadota, and Actinobacteriota. At the genus level, two groups were dominated with the *Ascomycota* and *Basidiomycota*. 1418 bacterial OTUs and 1192 fungal OTUs were observed in two groups. The SourceTracker analysis found that the average 49.27% bacterial community of BKS groups was from HDJ groups, and a middle 62.2% fungal community of BKS groups was from HDJ. Compared with the bacteria, the *F. bidentis* invasion had a more significant effect on the fungal community of the invasive soil. In conclusion, our results provided new insight into monitoring biodiversity protection in the YRD.

Key words: Invasive plants, High-throughput sequencing, Biodiversity protection, OTUs.

Introduction

With the acceleration of globalization, invasive plants have become an environmental issue of common global concern, posing a significant threat to environmental protection (Funk, 2015). They multiply and grow to form self-sustaining populations in the invaded areas, thereby changing and destroying the original ecological environment and ecological balance and ultimately affecting the environment (Gioria & Pyšek, 2015). In the invasion process, invasive plants often have a feedback adjustment effect on the soil ecosystem of the invaded area. This feedback adjustment effect will be more assertive, increasing the degree of invasion (Yang *et al*., 2016). The mutual feedback regulation between soils is important for plants' invasion. Plant traits and soil microbes affect carbon and nitrogen input and output processes. Previous studies mainly focused on the plant traits of surrogate plants among invasions. Meanwhile, the role of soil microorganisms was generally ignored in most research.

Previous found that invasive plants and their feedback seriously affected the colonization and dispersal of local habitats (Adkins & Shabbir 2014). On the one hand, plant litter and root exudates are released into the soil, providing a rich carbon source, thus changing the structure and function of soil microorganisms (Mehta *et al*., 2015). For example, *Acacia dealbata* changed soil nutrients through processes such as litter input carbon and biological nitrogen fixation, affecting the function of the soil microbial community after invading South Africa (Kamutando *et al*., 2019). The *Centaurea cyanus,* as an invasive plant, can secrete allelochemicals and affect the soil microbial community (Liao *et al*., 2015). On the other hand, soil microorganisms decompose nutrient elements that are difficult to absorb and utilize and accelerate material circulation invasive plants (Zubek *et al*., 2016). For example, *Alliaria petiolata* can increase the content of soil nitrogen, calcium, and other nutrients in the invaded land, improve the absorption and utilization of nutrients, form a soil environment that is beneficial to itself, and achieve successful invasion (Rodgers *et al*., 2008).

Flaveria bidentis (L.) Kuntze, a vicious weed that invaded China, was originally grown in South America, Brazil, and Argentina. Then gradually spread to other countries due to natural and human factors such as solid reproductive ability, ecological adaptability, and growth plasticity. Soil microbial communities were significantly affected by invasive plants (Zheng *et al*., 2019), which can affect the functional diversity of soil microbial communities in invasive areas (Shang *et al*., 2022a). As a key indicator reflecting the ecological characteristics of soil microorganisms, community diversity has become a hot spot and focus on ecological research (Li *et al*., 2021). In recent years, many research on the invasion of *F. bidentis* to soil ecosystems has been carried out on soil nutrient content (Chen *et al*., 2022), bacterial community diversity (Song *et al*., 2017), and fungal community diversity. The YRD has high species diversity and critical ecological status. However, there are many invasive species in the YRD, which have a significant impact on the protection of biodiversity. Therefore, we need to strengthen relevant research and protect it. Therefore, in this study, we studied the effect of *F. bidentis* on soil microorganism in the YRD.

Material and Method

Sampling collection and environmental data collection: The soil samples were collected from the YRD, Binzhou, Shandong Province. *F. bidentis* seriously invaded the sampling site, and the invasive time was about three years. The *F. bidentis* rhizosphere soil and bulk soils were collected in the study area. When collecting *F. bidentis* rhizosphere soil samples, we excavated the whole plant and removed the associated plants. Then, we used the brush to obtain the soil of *F. bidentis*. Meanwhile, we also collected the bulk soil. Three kinds of physical and chemical properties were analyzed in the present study, including the content of Total Organic Carbon (TOC), pH and Electrical conductivity (EC). The soil's physical and chemical properties were analyzed using previous detection methods (Shang *et al*., 2022b).

High-throughput sequencing of soil microbe: In this study, eight samples were obtained from two groups. The rhizosphere soil samples of *F. bidentis* were numbered HDJ group (HDJ1, HDJ2, HDJ3, HD4) and the bulk soil samples of *F. bidentis* were numbered BKS group (BKS1, BKS2, BKS3, BKS4). The V4 region and ITS region of microbe gene were amplified by PCR using the previous primers (Wu *et al*., 2016; Shang *et al*., 2022b). All sequencing data of eight samples was available in online database NCBI (accession number PRJNA913626 and PRJNA916286).

Data analysis: In the present study, the QIIME software (Version 1.9.1) calculated the Alpha diversity index (Bolyen, 2019), including the Chao1, ACE, Simpson, and Shannon. The variances of soil properties between the two groups were determined using the one-way analysis of variance (ANOVA). The QIIME software was also used for Beta diversity analysis to compare the similarity of different samples in terms of species diversity. All the figures were drawn by R (Version 3.4.4) (McMurdies & Holmes, 2013). The significantly different groups were analyzed by using the ANOVA, including the LDA effect size (LEfSe) and the SourceTracker analysis (Knights *et al*., 2011).

Results

Alpha diversity: The ACE (2010.15) and Chao1 (2009.52) bacterial index in HDJ groups was higher than that in BKS groups. which indicated a higher bacterial species abundance in *F. bidentis* rhizosphere soil (Fig. 1). The index of the Simpson (0.9973) and Shannon (9.74) is higher in the HDJ groups than in the BKS groups, indicating the bacterial species abundance and species evenness of non-rhizosphere soil was lower than in the rhizosphere soil of *F. bidentis* (Fig. 1). Meanwhile, the coverage ratios of all samples were 1.00.

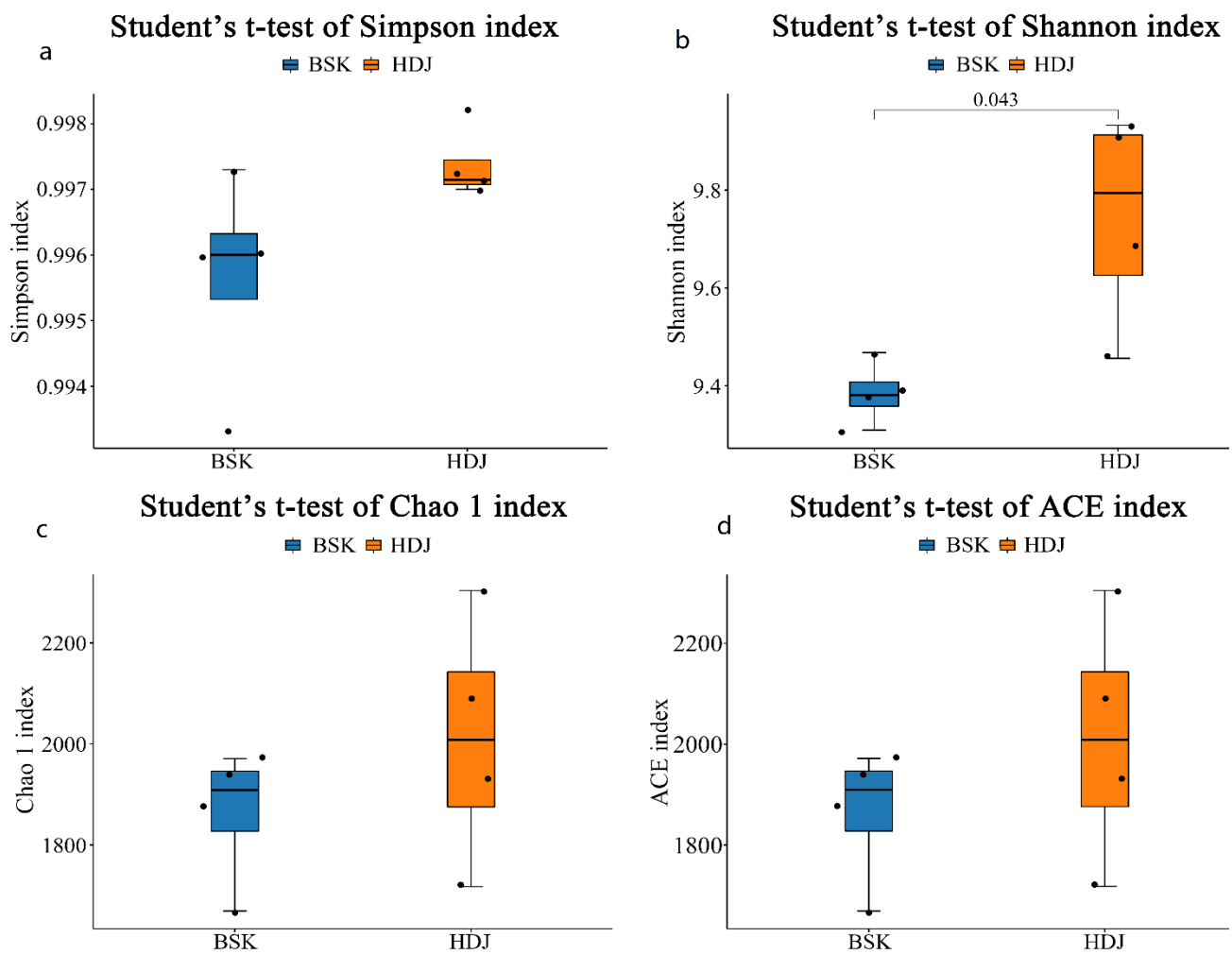

Fig. 1. Alpha diversity between the HDJ group and BSK group at bacterial level. HDJ:rhizosphere soil bacteria samples of *F. bidentis*; BSK: non-rhizosphere soil bacteria samples of *F. bidentis*. a: Simpson index; b: Shannon index; c: Chao1 index; d: ACE index.

For the fungi, the index of the ACE (1386.54) and Chao1 (1386.5) in HDJ groups was lower than that index of the ACE (1412.29) and Chao1 (1412.25) in BKS groups, indicating a higher fungal species abundance in *F. bidentis* non-rhizosphere soil (Fig. 2). The index of the Simpson (0.9892) and Shannon (8.467) is higher in the HDJ groups than in the BKS groups, indicating the fungal species abundance and species evenness of non-rhizosphere soil was lower than in the rhizosphere soil of *F. bidentis* (Fig. 2).

OTU analysis: For the bacteria, 6515 OTUs and 6158 OTUs were identified in the HDJ group and BSK group, respectively (Fig. 3). The unique OTUs number of HDJ group was higher than that of the BKS group. For fungal, a total of 7215 OTUs were identified in two groups. 4186 OTUs and 4221 OTUs were identified in the HDJ groups and the BSK groups, respectively. And 1192 common OTUs were identified between two groups.

The microbial community structure in different groups was analyzed in the present study. For bacteria, at the phylum level, two groups (HDJ and BSK groups) were dominated with the Proteobacteria, Acidobacteriota, Gemmatimonadota, and Actinobacteriota. The total relative abundance was 76.65% and 77.77%, respectively (Fig. 4). At the genus level, the BSK group were dominated with *Sphingomonas* and Subgroup_10; the HDJ groups were dominated with *Sphingomonas*, Subgroup_10, and *Bryobacter* (Fig. 4).

For fungi, the Ascomycota and Basidiomycota were dominant in HDJ and BSK groups at the phylum level (Fig. 4). Except for the unclassified species, the dominant fungi were *Fusarium, Candida*, *Cladosporium*, and *Russula* were dominant in HDJ and BSK groups at the genus level Fig. 4).

Beta diversity analysis: For the bacteria, the rates of PC1 and PC2 were 32.85% and 21.96%, respectively (Fig. 5). Our results showed that the samples within a group tend to cluster together. For the fungi, the PCA result showed that the rates of PC1 and PC2 were 62.21% and 12.68%. Moreover, the HDJ group tends to cluster together.

Correlation analysis: In this study, the pH and TOC were higher in the HDJ groups than in the BKS ($p>0.05$) (Table. 1). The EC was higher in the BKS groups than in the HDJ groups ($p>0.05$). For the bacteria, our results showed that the Actinobacteriota ($p<0.01$) and Proteobacteria ($p<0.05$) were significantly correlated with the EC, and the Gemmatimonadota was significantly correlated with the TOC ($p<0.05$) (Fig. 6). Through the analysis of three physicochemical properties and fungal composition, the Glomeromycota was significantly correlated with the TOC ($p<0.05$) (Fig. 6).

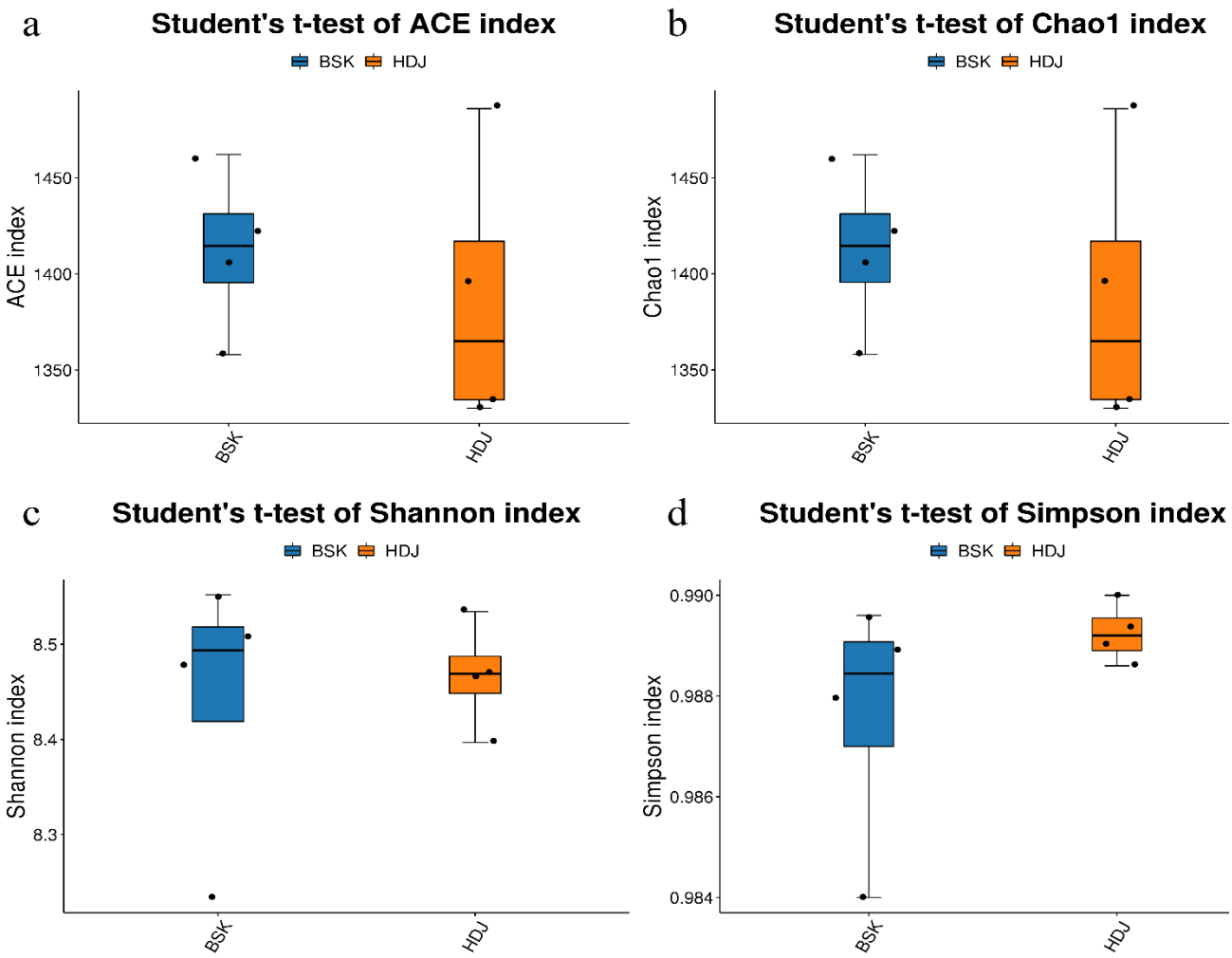

Fig. 2. Alpha diversity between the HDJ group and BSK group at fungal level. HDJ: rhizosphere soil fungal samples of *F. bidentis*; BSK: non-rhizosphere soil fungal samples of *F. bidentis*. a: ACE index; b: Chao1 index; c: Shannon index; d: Simpson index.

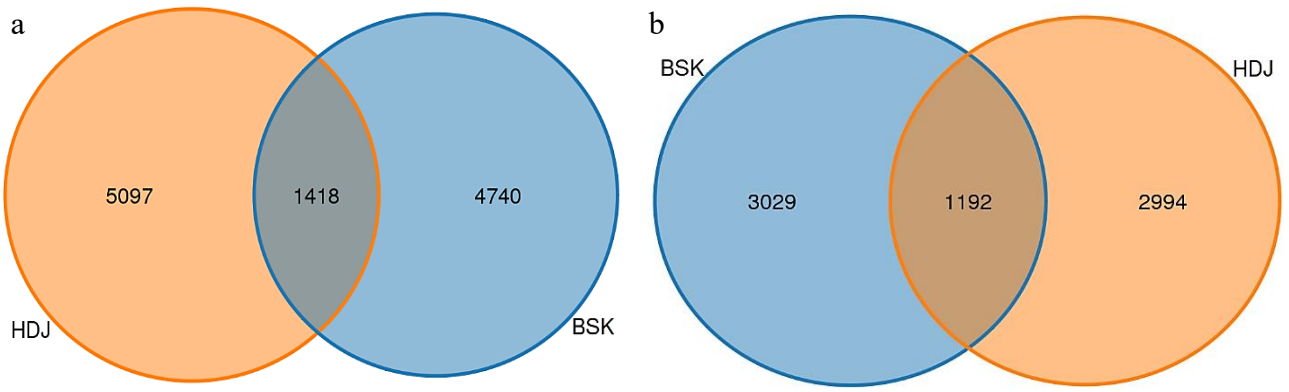

Fig. 3. OTUs analysis between two groups. HDJ: rhizosphere soil bacteria samples of *F. bidentis*; BSK: non-rhizosphere soil bacteria samples of *F. bidentis*. a: the bacterial Venn diagram between two groups; b: the fungal Venn diagram between two groups.

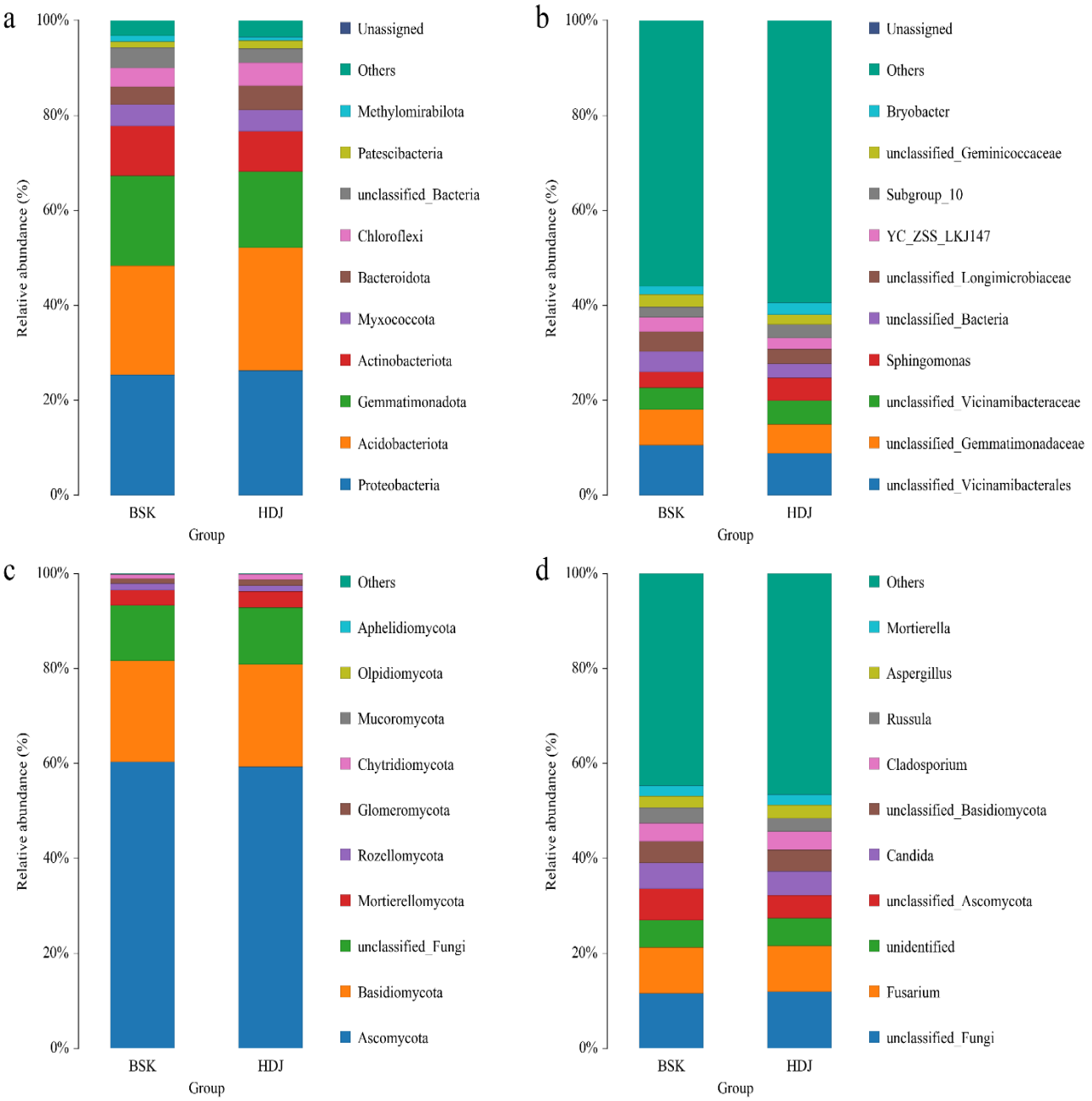

Fig. 4. The relative abundance of the bacterial communities in two groups. (a) bacterial communities at the phylum levels; (b) bacterial communities at the genus levels; (c) fungal communities at the phylum levels; (d) fungal communities at the genus levels. HDJ: rhizosphere soil bacteria samples of *F. bidentis*; BSK: non-rhizosphere soil bacteria samples of *F. bidentis*.

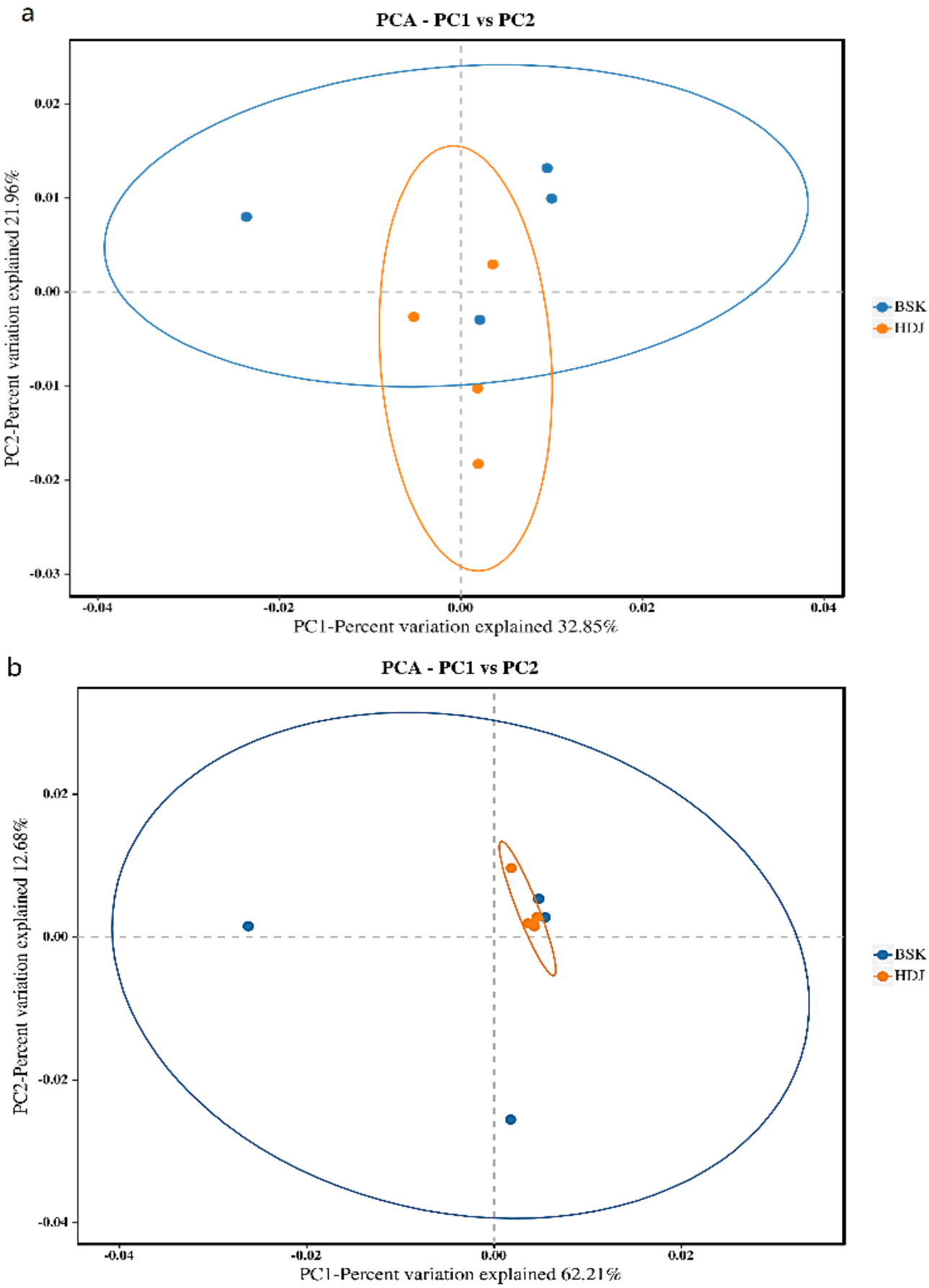

Fig. 5. The PCA analysis in bacterial and fungal community structure. Different colors represent different groups. (a) bacterial level; (b) fungal level. HDJ: rhizosphere soil bacteria samples of *F. bidentis*; BSK: non-rhizosphere soil bacteria samples of *F. bidentis*.

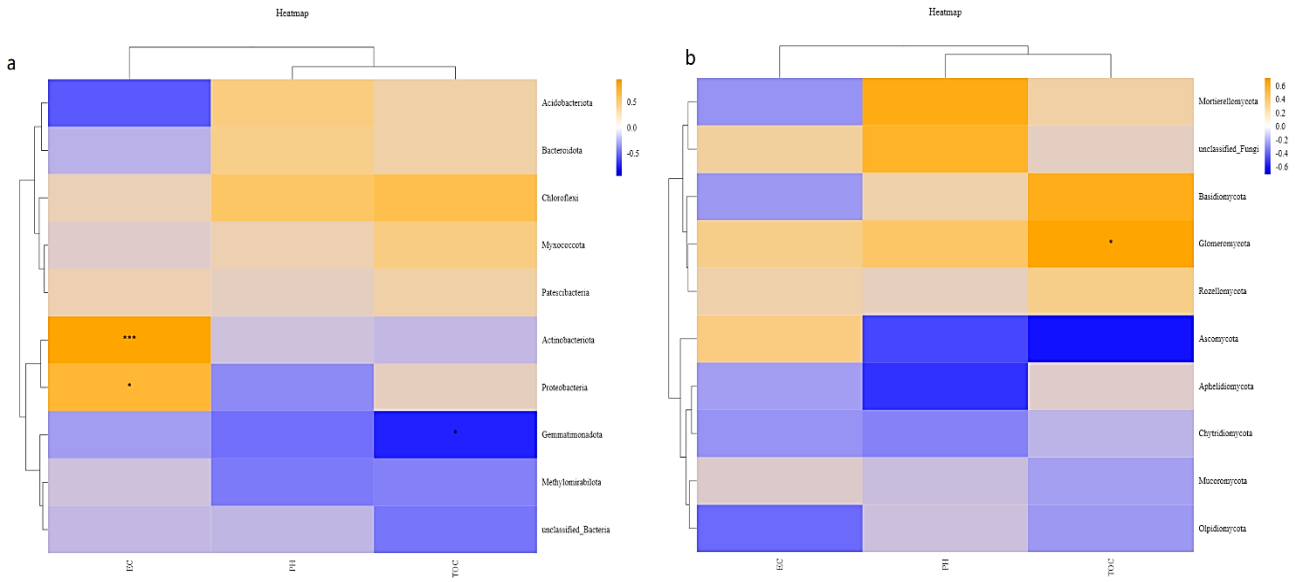

Fig. 6. Correlation analysis between two groups. a: the soil physicochemical properties and bacterial composition; b: the soil physicochemical properties and fungal composition.

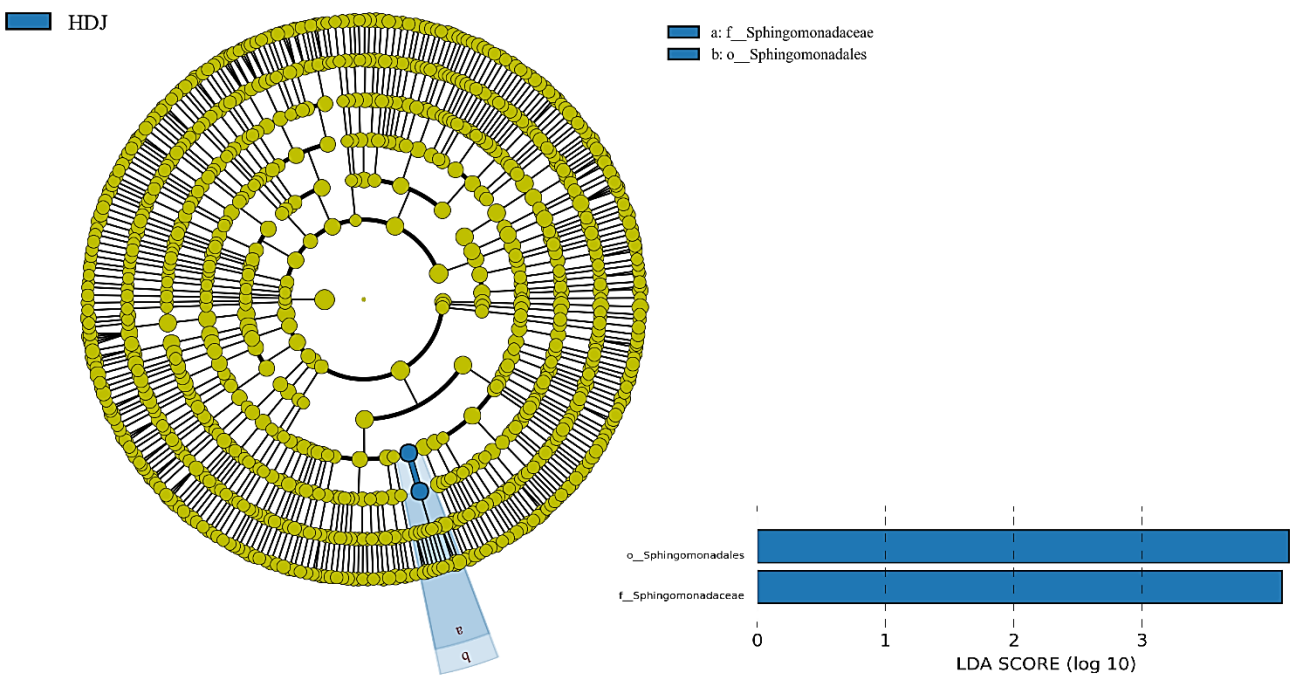

Fig. 7. LEfSe analysis between two groups. HDJ: the rhizosphere soil bacteria samples of *F. bidentis*; BKS: the bulk soil bacteria samples of *F. bidentis*.

Table 1. Soil physicochemical properties between two groups.

Groups	EC (μm/cm)	pH	TOC (g/kg)
HDJ	5.30 ± 1.60a	7.77 ± 0.06a	20.45 ± 1.23a
BKS	9.30 ± 5.17a	7.63 ± 0.06a	19.55 ± 0.64a

Note: Values are the means ± standard error (n=2). HDJ: the rhizosphere soil bacteria samples of *F. bidentis*; BKS: the bulk soil bacteria samples of *F. bidentis*

For the bacteria, the Sphingomonadaceae (at the family level) and Sphingomonadales (at the order level) were indicated to play an essential role in the HDJ group (Fig. 7).

The sources analysis in the target samples was explored based on the Bayesian algorithm (Fig. 8). For the bacteria, nearly half of the bacterial community in the BSK groups were from the HDJ group (average 49.27%). For the fungi, our results showed that more than half of the fungal community of the BSK groups were from HDJ groups (62.2%).

Discussion

Soil microbial community composition is closely related to aboveground plant community (Wardle *et al*., 2004). Invasive plants can significantly affect the native plant community (Wolfe & Klironomos, 2005). Plant invasion can change the essential biological characteristics of the natural community ecosystem (Jager *et al*., 2013).

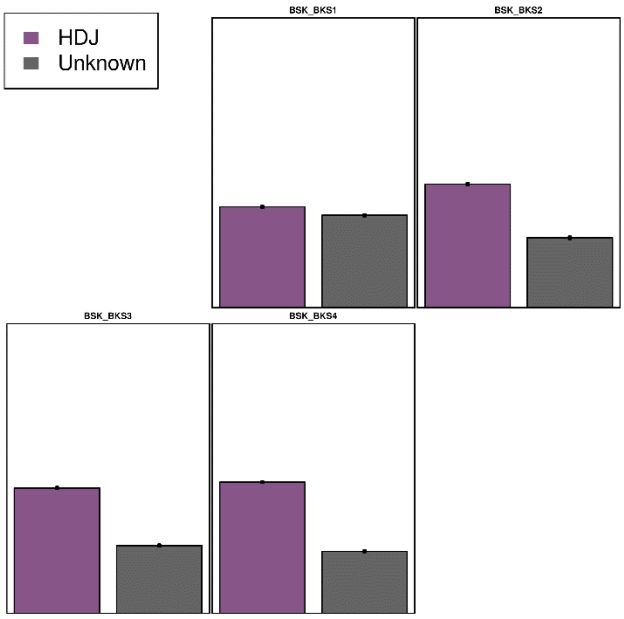

Fig. 8. SourceTracker analysis among two groups. HDJ: the rhizosphere soil bacteria samples of *F. bidentis*; BKS: the bulk soil bacteria samples of *F. bidentis*.

Our results showed that the bacterial alpha index in the HDJ groups was higher than that of the BSK groups. For the fungal alpha index, our results showed that the species abundance in *F. bidentis* rhizosphere soil was lower than that of *F. bidentis* non-rhizosphere soil. Thus, we speculated that the bacterial abundance and diversity in the rhizosphere of *F. bidentis are* higher than that in the non-rhizosphere, which is similar to the composition of bacterial diversity in the rhizosphere of many invasive plants (Torres *et al*., 2021). Moreover, the *F. bidentis* might improve its adaptability to the invasion environment by enhancing the number and variety of the bacteria. For bacterial communities, previous studies found that the dominant bacteria were Proteobacteria, Acidobacteriota, and Actinobacteriota in many invasive plants, such as the *Alternanthera philoxeroides* (He *et al*., 2022), *Parthenium hysterophorus* (Shang *et al*., 2023) and *Ambrosia trifida* (Li *et al*., 2022). Our results also found that the *F. bidentis* rhizosphere was dominated with Proteobacteria, Acidobacteriota, Gemmatimonadota, and Actinobacteriota. Meanwhile, the Acidobacteriota is widely distributed in various habitats and has essential functions (Kielak *et al*., 2016), such as using nitrogen sources, which play an important role in plant growth (Yang *et al*., 2016). The Acidobacteriota also plays an essential role in the *F. bidentis* rhizosphere. Actinobacteriota was also performed as one of the most significant taxonomic bacteria, identified as a plant growth-promoting rhizobacteria, playing a vital role in nutrient cycling and antimicrobial synthesis (Wang *et al*., 2022). Correlation analysis found that the Gemmatimonadota was significantly correlated with the TOC and the Actinobacteriota ($p<0.01$) were significantly correlated with the EC. Thus, Actinobacteriota might also be necessary for the invasion of *F. bidentis*. For the fungal communities, previous studies found that Ascomycota and Bipolaris were dominant in the invasive species (Wang *et al*., 2022). Moreover, Ascomycota was one of the most abundant phyla in many invasive sites (Wang *et al*., 2018). In the present study, our results showed that Ascomycota is dominant in the *F. bidentis* rhizosphere. The Ascomycota might be beneficial to the growth and competitiveness of the *F. bidentis* invasion (Wang *et al*., 2022). The OUT results showed that 1418 bacterial OTUs and 1192 fungal OTUs were observed in all the groups. The SourceTracker analysis found that the average 49.27% bacterial community of BKS groups was from HDJ groups, and an average 62.2% fungal community of BKS groups was from HDJ. Compared with the bacteria, the *F. bidentis* invasion had a more significant effect on the fungal community of the invasive soil. The LEfSe analysis of the Sphingomonadaceae (at the family level) and Sphingomonadales (at the order level) were important for the HDJ group. A previous study found that Sphingomonadaceae was significantly more abundant in the rhizosphere of sugar beet seedlings upon fungal invasion (Chapelle *et al*., 2016). Meanwhile, Sphingomonadaceae was identified as the most abundant responsive taxa in the rhizosphere after pathogen invasion (Deng *et al*., 2022). Thus, Sphingomonadaceae might play an essential role in *F. bidentis* invasion. Moreover, Sphingomonadales was well-known as beneficial bacteria and closely associated with plant root exudates (Ge *et al*., 2022).

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