Infection of *Plasmodiophora brassicae* changes the fungal endophyte community of tumourous stem mustard roots as revealed by high-throughput sequencing and culture-dependent methods

Abstract

Diverse fungal endophytes live in plants and are shaped by some abiotic and biotic stresses. Plant disease as particular biotic stress possibly gives an impact on the communities of fungal endophytes. In this study, clubroot disease caused by an obligate biotroph protist, *Plasmodiophora brassicae*, was considered to analyze its influence on the fungal endophyte community using an internal transcribed spacer (ITS) through high-throughput sequencing and culture-dependent methods. The results show that the diversity of the endophyte community in the healthy roots was much higher than the clubroots. Ascomycota was the dominant group of endophytes (including *Phoma*, *Mortierella*, *Penicillium*, etc.) in the healthy roots while *P. brassicae* was the dominant taxon in the clubroots. Hierarchical clustering, principal component analysis (PCA) and principal coordinates analysis (PCoA) indicated significant differences between the endophyte communities in the healthy roots and clubroots. Linear discriminant analysis effect size (LefSe) analysis showed that the dominant genera could be regarded as potential biomarkers. The endophyte community in the healthy roots had a more complex network compared with the clubroots. Also, many plant pathogenic *Fusarium* were isolated from the clubroots by the culture-dependent method. The outcome of this study illustrates that *P. brassicae* infection may change the fungal endophyte community associated with the roots of tumourous stem mustard and facilitates the entry of soil pathogen into the roots.

Introduction

Fungal endophytes have a close relationship with the host plants and they live in their tissues [1,2]. They provide many ecological and physiological advantages to their hosts, such as growth promotion [3], resistance to plant pathogens and adaptability to various abiotic stresses such as temperature, pH, and osmotic pressure as well as biotic stresses [4-6]. Similarly, the fungal endophyte communities are also affected by abiotic and biotic stresses [7]. Plant disease as particular biotic stress results in significant changes in the physiology of the plant. These changes and pathogen itself may affect the diversity and composition of the fungal endophyte inhabiting the plant tissues and their interactions with their host plant [8-10].

Clubroot is a severe disease of cruciferous crops such as cabbage and cauliflower, which is caused by the biotrophic *Plasmodiophora brassicae* Woronin [11]. The infected root cells undergo abnormal cell division and enlargement resulting in the formation of spindle-like, spherical, knobby or club-shaped swellings [12]. The root galls significantly alter the morphology, development, and physiology of the diseased plants [13]. Moreover, *P. brassicae* consumes carbohydrates from the viable cells in gall, and make gall into a sink for nutrient substances [14,15].

Tumourous stem mustard (*Brassica juncea* var. *tumida*) is an economical and nutritionally important vegetable crop widely grown in Fuling County, a district in Chongqing Municipality, China. Clubroot is one of the most harmful diseases of tumourous stem mustard causing substantial economic damage [16]. The diseased tumourous stem mustard shows small plants and swollen roots and finally dies due to root rot during the late plant growth stage in the field, although *P. brassicae* is an obligate parasite. Moreover, many pathogenic fungi such as *Fusarium* sp. are isolated from the clubroots, which indicates that the community of fungal endophyte may change during the infection of *P. brassicae*. However, the specific

alteration in the community of fungal endophyte is still unclear.

In this context, the objectives of this study are, (1) to examine the diversity and composition of the fungal endophyte community in the roots of tumourous stem mustard (2) to demonstrate the changes in the community of fungal endophyte in the clubroots of tumourous stem mustard caused by *P. brassicae* compared to healthy roots .

Materials and methods

Sample collection

P. brassicae-infected tumourous stem mustard roots were obtained from three fields (February 2, 2017) in Fuling (29.21° N, 106.56° E) where clubroot disease was observed during the last 20 years. Roots were collected from the plants at the harvest-stage. The roots were classified as either R (healthy roots, no clubroot symptoms) or C (diseased roots, swollen clubroot symptoms). From one field, 30 plants including 15 R and 15 C samples were randomly selected and formed as one group. By this, three groups contained 90 plants from three fields and were referred to R1, C1, R2, C2, R3, and C3. The roots were washed with tap water to remove the soil particles. The healthy roots with a diameter of 0.5 cm from undiseased plants and clubroot galls with a diameter of 1 cm from diseased plants were cut off, and then sterilized by 70 % (v/v) ethanol for 40 s, followed by 4 % (w/v) sodium hypochlorite for 60 s, and finally washed three times using sterile distilled water. The surface sterilized healthy roots and galls peeled with a sterilized razor were divided into three portions. One portion was used to extract genomic DNA by cetyltrimethylammonium ammonium bromide (CTAB) DNA extraction method. The concentration and purity of DNA were monitored on 1% agarose gel. One portion was made into paraffin section, dyed with sarranine and observed under an optical microscope. Another portion was used to isolate endophytic

67 fungi by the culture-dependent method.

68 **Isolation of endophytic fungi and its taxonomic identification**

69 Three types of media, i.e. potato dextrose agar (BD Difco), rose bengal medium (BD Difco), and Czapek
70 medium (BD Difco) were used for the isolation of endophytic fungi. The surface sterilized healthy roots,
71 or clubroot samples were cut into tissue blocks of 5 mm x 5 mm, and were planted on a medium at 25
72 ◦C in the dark. When mycelia appeared, they were transferred into another medium for purification. A
73 total of 156 fungal isolates were then identified based on the ITS sequence data. Mycelia of the fungal
74 isolates were ground with liquid nitrogen in a sterile mortar, and Genomic DNA from all the fungal
75 isolates was extracted using DNA extraction kit (TIANGEN Co. Ltd., Beijing, PR China). The internal
76 transcribed spacer (ITS) region was amplified with universal primers (ITS1 and ITS4) [17]. PCR mixture
77 contained 12 μL of 2× Taq PCR Mix (TIANGEN Co. Ltd., Beijing, PR China), 1 μL DNA template, 1
78 μL of each primer and 8 μL double distilled water. The PCR reaction conditions were as follows: initial
79 pre-heating at 94 ◦C for 3 min, 30 cycles of 94 ◦C for 30 s, 58 ◦C for 30 s, 72 ◦C for 30 s, and a final
80 extension at 72 ◦C for 10 min. PCR products were sequenced by 3730 sequencer (Majorbio Tech Co.
81 Ltd., Beijing, PR China). The ITS sequence was aligned in the NCBI database
82 (http://www.ncbi.nlm.nih.gov/) using the BLAST program. The highest hits (identity values higher than
83 97%) were regarded as the taxonomy of fungal isolates. The relative abundances were calculated
84 according to the taxonomy of fungal isolates.

85 **High-throughput ITS Sequencing**

86 ITS fragments were used as marker genes for high-throughput sequencing, and the primers of ITS
87 fragments target most fungal groups, as well as other eukaryotes. The ITS fragments were amplified by
88 PCR for barcoded pyrosequencing using the primers ITS1 and TS2 [18]. The employed PCR conditions

89 were: 95 °C for 2 min (one cycle), 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s (25 cycles), and
90 72 °C for 5 min (one cycle). The sequencing was performed using an Illumina MiSeq sequencer (Asbios
91 Technology Co., Ltd, China).

92 **Processing of Bioinformatics and Data Analysis**

93 For diversity analyses, the bioinformatics analysis pipeline was conducted on the free online platform,
94 Majorbio I–Sanger Cloud Platform (http://www.i-sanger.com). First, the raw sequences were processed
95 using the QIIME package (v1.8) [19]. The low-quality sequences, such as primer and barcode sequence
96 mismatch, and PCR-based or sequencing errors and chimeras, were removed. The taxonomy of each
97 operational taxonomic unit (OTU) representative sequence was performed using Unite (Release 7.2) and
98 ITS of *P. brassicae* under the threshold of 97% identity [20,21]. The Shannon index, Simpson index, and
99 rarefaction curves were calculated to evaluate the α-diversity. Relative abundance of endophyte was
100 calculated at phylum, genus and OTU levels. Analysis of variance (ANOVA) of the Shannon and
101 Simpson indices was obtained by SPSS 16.0 and the relative abundance was used to assess the differences
102 in the endophyte communities between the healthy roots and clubroots. For the evaluation of β-diversity,
103 hierarchical cluster dendrograms (Bray-Curtis distance dissimilarities) were constructed according to the
104 composition of OTU [22]. An unweighted UniFrac principal component analysis (PCA) and Principal
105 coordinates analysis (PCoA) were performed using R 3.1.1 statistical software [23]. The differentially
106 abundant genera between the healthy roots and clubroots were identified for finding the biomarker by
107 Discriminant Analysis Effect Size (LEfSe) software [24]. Co-occurrence network analysis was
108 conducted to reveal the relationship among the top 30 OTUs within the endophyte communities by
109 Networkx software [25]. Pearson's rank correlation coefficients between OTUs were calculated, and
110 only the correlations with $r > 0.5$ were selected to construct the network.

111

112 **Results**

113 **Symptoms of Clubroot and Detection of *P. brassicae***

114 Healthy roots were not swollen, and the lateral roots appeared normal, whereas clubroots were swollen
115 and few lateral roots were observed (Fig. 1A). Abundant resting spores were found to be in the cells of
116 clubroots (Fig. 1B), but no resting spores were observed in the healthy roots (Fig. 1C).

117 **Analysis of α-diversity**

118 High-quality sequences of ITS were produced by the Miseq platform. The raw sequencing data for ITS
119 sequencing were deposited at the Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra)
120 under an accession number, SRP136514. The statistical formation of the sequences is shown in Table
121 S1. According to the taxonomy of the sequence and abundance (Table S2), the composition of the
122 endophyte communities was analyzed. Rarefaction curves analysis confirmed that the number of
123 observed OTUs increased asymptotically with an increase in the reads (Fig. 2A). Both a higher Shannon
124 index and Simpson index of the endophyte community were observed in the healthy roots than that in
125 the clubroots (Fig. 2B), suggesting a higher diversity of the endophyte community in the healthy roots.

126 Ascomycota was the dominant taxon (a relative abundance ranging from 76.3 to 78.5%) of the
127 endophyte community in the healthy roots followed by Zygomycota (RA, 15.5 to 19.9%), while
128 Cercozoa (RA, 92.0 to 94.3%) was the most dominant taxon of the endophyte community in the clubroots
129 followed by Ascomycota (RA, 5.6 to 7.6%) (Fig. 3A). The abundance of Basidiomycota and unclassified
130 endophytic fungi was higher in the healthy roots compared to clubroots.

131 At the genus level, a total of 40 and 27 genera were detected in the healthy roots and clubroots,
132 respectively. In the healthy roots, *Phoma* was the dominant genus with an RA of 19.15%, followed by

133 *Mortierella* (17.09%), *Penicillium* (11.49%), *Pseudallescheria* (10.75%), *Tetracladium* (9.98%), and
134 *Fusarium* (7.88%) (Fig. 3B). In the clubroots, *Plasmodiophora* was the dominant taxon (93.28 %)
135 followed by *Fusarium* (5.52 %) (Fig. 3B). Circos figure confirmed that *Plasmodiophora* was the most
136 abundant and was found only in the clubroots, whereas *Phoma*, *Mortierella*, and *Penicillium* were
137 predominant in the healthy roots (Fig. 3C).

138 A total of 66 and 40 OTUs were observed in the healthy roots and clubroots, respectively. Heatmap
139 showed that OTU268 was dominant in the endophyte community associated with clubroots, whereas
140 OTU106, OTU153, OTU192, OTU197, OTU114, OTU126, and OTU185 dominated the healthy roots
141 (Fig, S1).

142 **Analysis of β-diversity**

143 Hierarchical clustering analysis based on Bray-Curtis distance dissimilarities revealed that the
144 endophyte communities in the healthy roots and clubroots clustered in the two branches (Fig. 4A).
145 UniFrac-weighted PCA based on the composition of OTU showed variations between the healthy roots
146 and clubroots with the first two axes indicating 81.08 and 11.71 % of the total variation (Fig. 4B). PCoA
147 plot also clearly demonstrated similar results of PCA with the first two axes showing 99.34 and 0.62%
148 of the total variation (Fig. 4C). The endophyte community in the healthy roots was clustered on the right
149 side of the PCA and PCoA plot while the endophyte community in the clubroots was clustered on the
150 left side, indicating a clear separation between the endophyte community in R and C samples.

151 From the results of LEfSe, a significantly different taxa were found out between the two communities.
152 At the genus level, *Phoma*, *Mortierella*, *Penicillium*, unclassified Pleosporales, etc. were enriched in the
153 healthy root samples (Fig. 5A) and abundant *Plasmodiophora* in the clubroot samples possessed high
154 values of linear discriminant analysis (LDA) (Fig. 5B). These taxa with different abundance can be

155 regarded as potential biomarkers (LDA>3, P<0.05). Furthermore, 15 most abundant genera of the two
156 endophyte communities were compared by Student's t-test, where significant differences were observed
157 (Fig. S2). For instance, *Plasmodiophora* was significantly more abundant in the clubroots while all other
158 genera except *Fusarium* were significantly more abundant in the healthy roots.

159 **Network Analysis**

160 The endophyte community in the healthy roots had two centers with a complex network structure (28
161 nodes and 336 edges) (Fig. 6A) while the endophyte community in the clubroots had three centers with
162 a less complex network structure (18 nodes and 161edges) (Fig. 6B). Most of the nodes belong to
163 Ascomycota in both the healthy roots and clubroots networks and had the highest number of correlations
164 with other taxa. The node of the Cercozoa (i.e., *Plasmodiophora*) only appeared in the clubroot network
165 and had the highest percentage. When the relationship between *Plasmodiophora* and other fungi was
166 analyzed, it was found that 10 OTUs had a significant correlation with *Plasmodiophora* (3 OTUs had a
167 positive correlation, and 7 OTUs had negative correlation) (Table S3).

168 **Culturable endophytic fungi**

169 The same samples for Miseq sequencing were also used to isolate endophytic fungi by the culture-
170 dependent method. At the genus level, *Mortierella*, *Sarocladium*, *Phoma*, *Penicillium*, *Plectosphaerella,*
171 and *Fusarium* were obtained (Fig. 7A). *Fusarium* (a relative abundance of 81.4%) was the predominant
172 group in the clubroots, among which the following were present: *F. graminearum* (RA, 5.5 %), *F.*
173 *oxysporum* (RA, 28.8 %), *F. solani* (RA, 24.0 %), *F. asiaticum* (RA, 8.2 %), and *Fusarium* sp.
174 (RA 14.4 %). In the healthy roots, *Mortierella* was the main group with an RA of 36.2% followed
175 by *Phoma*, *Penicillium*, *Fusarium,* etc. (Fig. 7B).

176 **Discussion**

In this study, a high diversity of fungal endophyte in the healthy roots of tumourous stem mustard has been found out using high-throughput sequencing. Our results agree with the early reports that the plants can harbor a diversity of endophytic fungi in their healthy tissues, especially in the root system which is considered to be the most suitable habitat for endophytic fungi [26,27]. Ascomycota is reported to be the most common group of endophytic fungi [28], suggesting that they are suitable for the ecological niche of plant tissue. Zhao also reported Ascomycota as the dominant group of endophytic fungi in the roots of oilseed rape (*Brassica napus*) [29]. Also in this study, Ascomycota has been found to be the dominant taxon in the healthy roots of tumourous stem mustard. In the phylum Ascomycota, *Phoma*, *Mortierella*, *Penicillium,* and *Fusarium* were the common fungal genera in the healthy roots of tumourous stem mustard. Arie et al. reported that *Phoma glomerata* produces epoxydon and inhibits clubroot disease, demonstrating that *Phoma* in the healthy roots of tumourous stem mustard may perform a similar function [30,31]. *Mortierella* was also detected in the roots of oilseed rape using high-throughput sequencing [29]. Melo et al. confirmed that the endophytic *M. alpina* in the moss Schistidium antarctici produces antibiotics, antioxidant substances, and polyunsaturated fatty acids, which improve the environmental suitability of the plant host [32]. Wani et al. also found that endophytic *M. alpina* promotes the biosynthesis of Crocus apocarotenoid and enhances environmental stress tolerance [33]. These results reflect that the endophytic *Mortierella* is a benefit to the plant, which indicates that *Mortierella* in tumourous stem mustard is profitable to the host. Marinho et al. and Lin et al. both reported that endophytic *Penicillium* produces active polyketides [34,35], reflecting that *Penicillium* in tumourous stem mustard may produce similar substances.

Worldwide *Fusarium* is considered to be one of the most ubiquitous groups of fungi which can survive in a wide range of plants, environments, and climates [36]. In this study, some OTUs identified as

199 *Fusarium* had a relatively high abundance in both the healthy roots and clubroots, reflecting the dominant
200 presence of *Fusarium* in tumorous stem mustard. Previous studies show that most of the *Fusarium*
201 species are nonpathogenic while only a small number are pathogenic [37]. Some OTU identified as
202 *Fusarium* in the healthy roots perhaps were nonpathogenic because of the absence of wilt symptoms.
203 OTU244 and OTU287 assigned as *F. solani*, a common soil-borne pathogen, were only and mainly found
204 in the clubroots and these populations may be pathogenic. Moreover, *F. solani* is the most fungal isolate
205 obtained by the culture-dependent method (Fig. 7A). Also, other pathogenic *Fusarium* such as *F.*
206 *graminearum*, *F. oxysporum,* and *F. asiaticum* also isolated from the clubroots rather than the healthy
207 roots, suggesting that *Plasmodiophora* infection many facilitate *Fusarium* to enter into the diseased roots.
208 The presence of a plenty of pathogenic *Fusarium* in the clubroots maybe the reason for the death of
209 tumourous stem mustard during the late plant growth stage in the field. In clubroots, *Plasmodiophora*
210 was the extremely abundant genus, although not belonging to fungi, amplified by PCR with ITS primers
211 for fungi and other eukaryotes. Zhao et al. also found that *Plasmodiophora* mainly enriched in the
212 clubroots of *Brassica napus* by high throughput sequencing using the same primers [29].

213 In general, the healthy plant tissues harbor a more diverse community of endophytic fungi than
214 diseased plant tissues [29,38]. Also, the endophyte community in the healthy roots of tumourous stem
215 mustard had significantly higher Shannon and Simpson indices compared with the clubroots. Also, a
216 higher number of genera and OTUs were obtained from the healthy roots than from the clubroots. The
217 endophyte communities in the healthy roots and clubroots also differed in the α- and β-diversity. The
218 marked difference in the composition of the endophyte community at the genus level between the healthy
219 roots and clubroots as revealed by LEfSe and Student's t-test showed that the dominant genera as
220 biomarkers caused differences in the two communities. These substantial discrimination in the dominant

221 genera was derived from the infection of *P. brassicae*. Pathogens may compete with the endophytic fungi
222 for space and nutrients in the same niche within the plant tissue [39]. We presume that a decrease in the
223 diversity of the fungal endophyte community in the clubroots of tumourous stem mustard has two causes.
224 First, the physiological changes and the formation of clubroots induced by *P. brassicae* may affect the
225 availability of nutrients for endophytes. Second, in clubroots, the resting spores of *P. brassicae* are
226 produced in the galls of diseased plants and may fill the gall cells. This may restrict the available space
227 for the endophytes. The phenomenon of a decrease in the diversity of endophytic fungi has been observed
228 in many diseased plant species [29,40,41].

229 In this study, some plant pathogenic fungi were detected from the clubroots, such as *F. solani*, but
230 not from the healthy roots of tumourous stem mustard. Similarly, Zhao et al. isolated many soil-borne
231 pathogenic fungi, including *Fusarium*, *Gibberella*, *Alternaria*, *Sclerotinia*, *Leptosphaeria,* and
232 *Cylindrocarpon*, from the oilseed rape roots infected with *P. brassicae* [29]. This multi-species
233 aggregation involving *P. brassicae* and other pathogenic fungi is similar to disease complexes containing
234 plant-parasitic nematodes and soil-borne pathogens [42]. The cuticle of clubroots is cracked, thereby
235 providing entry points for pathogens, even for opportunistic pathogens. Additionally, the cracked cuticle
236 aggravates the leaking of nutrients, such as amino acids and carbohydrates, into the rhizosphere soil
237 which may attract soil-borne pathogens and increase the risk of infection by these pathogens [43]. Root-
238 knot nematodes may form disease complexes with *Fusarium* species such as *F. oxysporum* and *F. solani*
239 [44]. In this study, the abundant presence of *F. solani* in the clubroots indicates that this pathogen and *P.*
240 *brassicae* may form a disease complex, which may make it more difficult to control the clubroot disease.
241 Further studies are necessary to clarify the potential existence of this disease complex. Besides, fungi
242 such as *C. cupreum*, *Phoma* sp. *Pochonia* sp. and *Myrmecridium schulzeri* were harvested and were

243 reported as biocontrol fungi [45-47], reflecting that these fungi may help the host to resist the infection
244 of *P. brassicae*.

245 Endophytic fungi in the plants must deal with many interactions and build a balanced network [48].
246 In this study, the endophyte community network in the healthy roots of tumourous stem mustard was
247 observed to be more complicated than in the clubroots, indicating a balanced network of interactions
248 among the fungal endophyte community in the healthy roots. In the clubroots, *P. brassicae* dominated
249 the endophyte community which was out of balance showing a weak network of interactions. In general,
250 endophytes are more diverse and abundant than the pathogens in a healthy plant microbiome, and *vice*
251 *versa* in a diseased plant microbiome [49].

252

253 **Conclusions**

254 In conclusion, the obtained data from this study showed that the fungal endophyte community in the
255 clubroots are markedly different from the healthy roots in terms of alpha and beta diversity, suggesting
256 that the infection of *P. brassicae* changes the fungal endophyte community in tumourous stem mustard
257 roots. Future work should involve identifying the pathogenicity of *F. oxysporum* and *F. solani* on
258 tumourous stem mustard. Moreover, some endophytic fungi with biocontrol activity against *P. brassicae*
259 could also be considered in the evaluation.

260

261 **Figure legends**

262 Fig 1. **Healthy roots and clubroots of tumourous stem mustard caused by the infection of *P.***
263 ***brassicae*** (A) roots of a healthy plant (left) and a diseased plant (right) (B) resting spores (pots) in the
264 root cells of cluboorts coloured with fast green (C) the cell of healthy roots.

Fig 2. **Rarefaction curves (A) and diversity indices (B) of the endophyte communities associated with the healthy roots and clubroots of tumourous stem mustard infected with *P. brassicae***. R, healthy roots. C, clubroots. ** differences at 0.01 level.

Fig 3. **Relative abundance (%) of the endophyte at phylum (a), genus (B) and Circos figure at the genus level (C) in the healthy roots and clubroots of tumourous stem mustard infected with *P. brassicae***. R, healthy roots. C, clubroots.

Fig 4. **Hierarchical clustering analysis (a) UniFrac-weighted PCA (B) and PCoA (C) of the endophyte communities associated with the healthy roots and clubroots of tumourous stem mustard infected with *P. brassicae***. R, healthy roots. C, clubroots.

Fig 5. **LefSe analysis (A) The cladogram diagram shows the taxas with marked differences in the two endophyte communities**. Red and green indicate different groups, with the classification of taxas at the level of phylum, class, order, family, and genus shown from inside to the outside. The red and green nodes in the phylogenetic tree represent taxas that play an important role in the two endophyte communities, respectively. Yellow nodes represent taxas with no significant difference. (B) Species with the significant difference that have an LDA score higher than the estimated value; the default score is 3.0. The length of the histogram represents LDA score; i.e., the degree of influence of taxas with a significant difference between different groups. R, healthy roots. C, clubroots.

Fig 6. **Co-occurrence network analysis of the two endophyte communities within the healthy roots and clubroots of tumourous stem mustard infected with *P. brassicae*** (A) Healthy roots (B) Clubroots. Each node represents taxa affiliated at the OTU level, and the size of the nodes represent an average abundance of OTU. The lines represent the connections between each OTU. A red line indicates a positive correlation. whereas a green line indicates a negative correlation.

Fig 7. The endophytic fungi isolated from the clubroots (A) and healthy roots (B) by the culture-dependent method.

Supporting information

Table S1 Sequence information of each sample. R, healthy roots. C, clubroots.

Table S2 Taxonomy and distribution of the OTUs. Taxonomy at Phylum, Class, Order, Family, Genus, Species, and OTU level. R, Healthy roots. C, Clubroots. The number in the table cell is the number of sequences of each OTU.

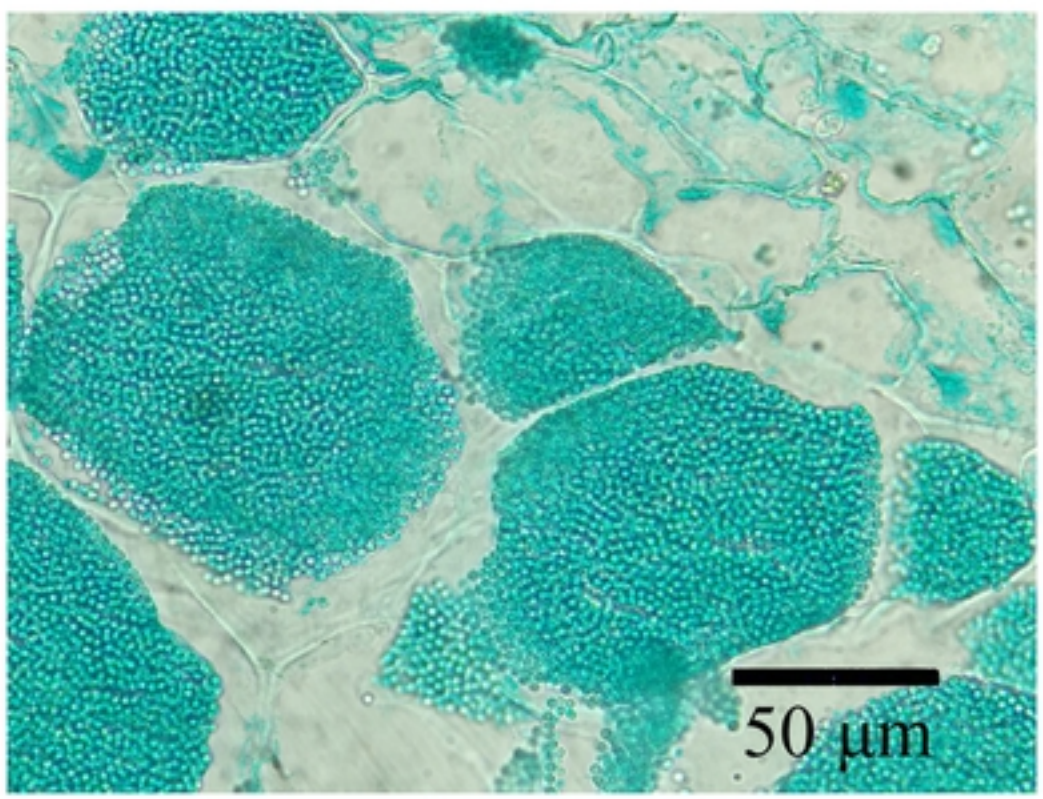
Table S3 Pearson's correlation relationships between *Plasmodiophora* and OTUs showed in the network.

Fig S1Heatmap of the 50 most abundant OTU from R and C samples. R, healthy roots; C, clubroots.

Fig S2 Student's t-test bar plot of the endophyte communities at the genus level in the healthy roots and clubroots of tumourous stem mustard infected with *P. brassicae*. $p<0.05$*, $p<0.01$**, $p<0.001$***. R, healthy roots. C, clubroots.

a

b



c

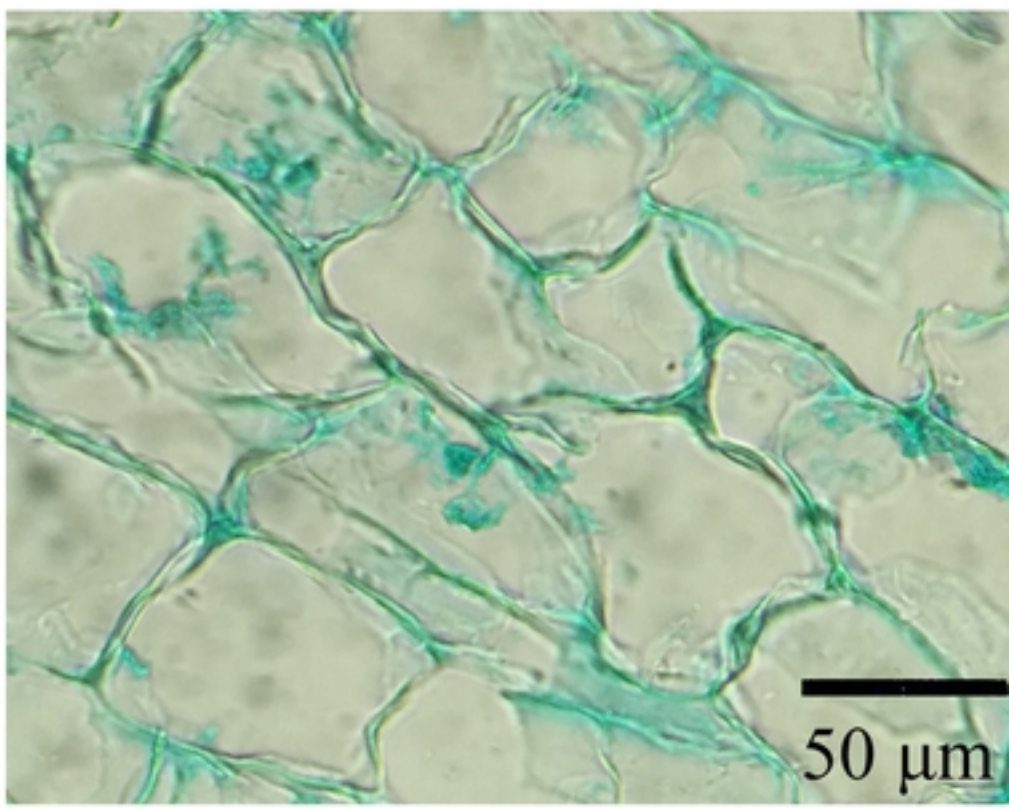
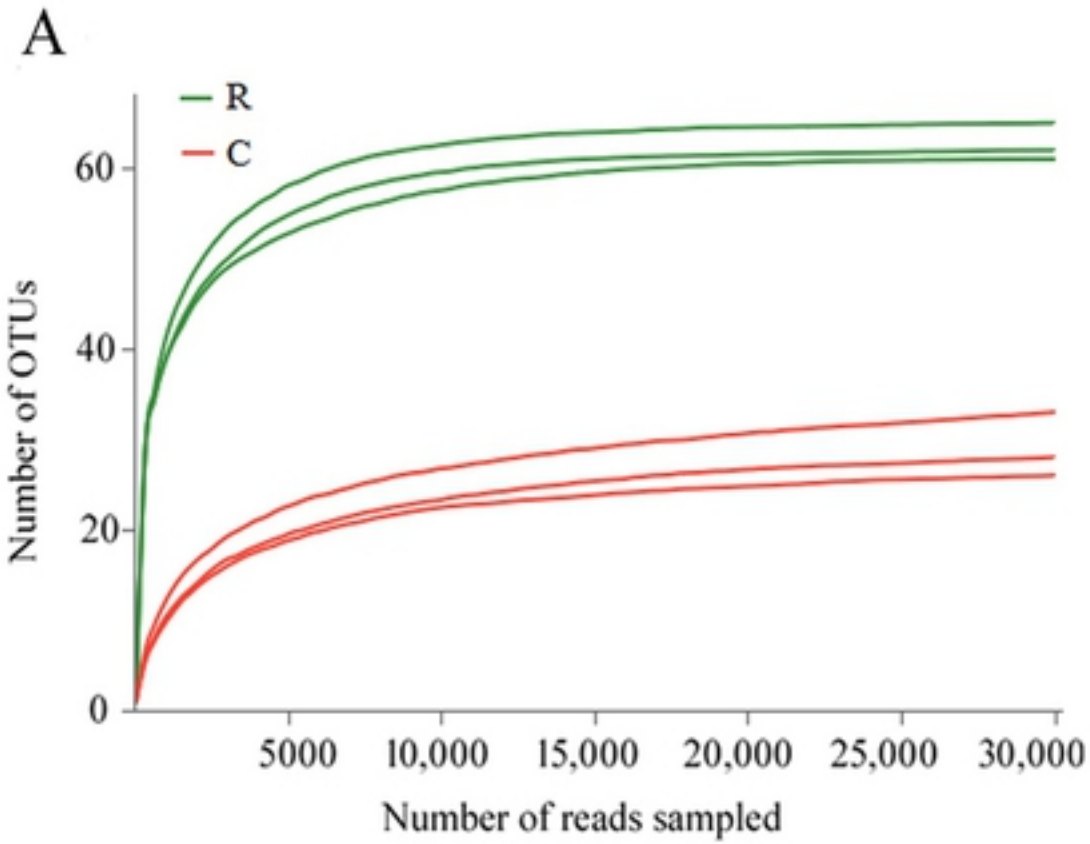
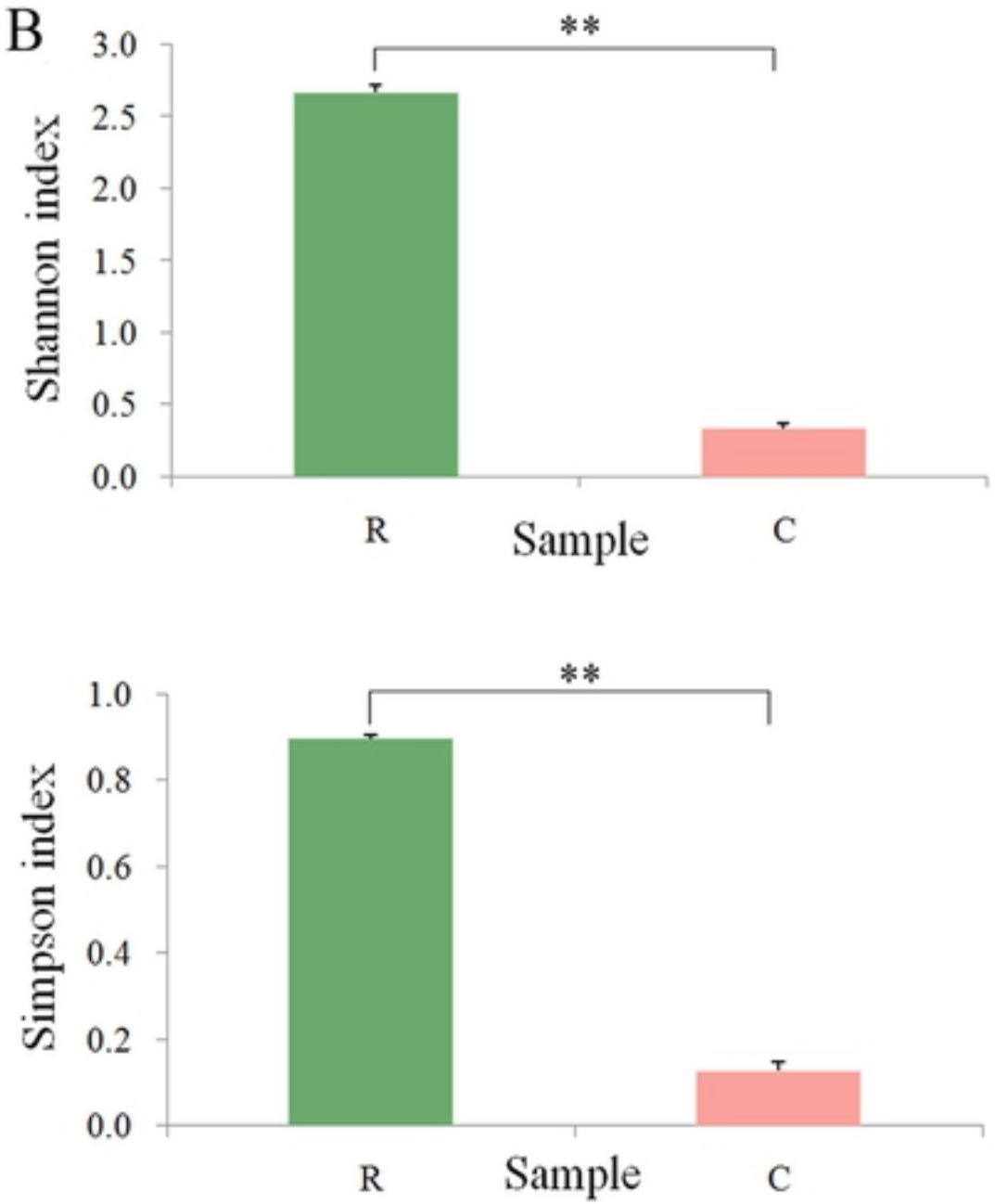


Figure1


A
R
C
Number of OTUs
0
20
40
60
5000
10,000
15,000
20,000
25,000
30,000
Number of reads sampled


B
**
Shannon index
3.0
2.5
2.0
1.5
1.0
0.5
0.0
R
Sample
C
**
Simpson index
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0.8
0.6
0.4
0.2
0.0
R
Sample
C

Figure2

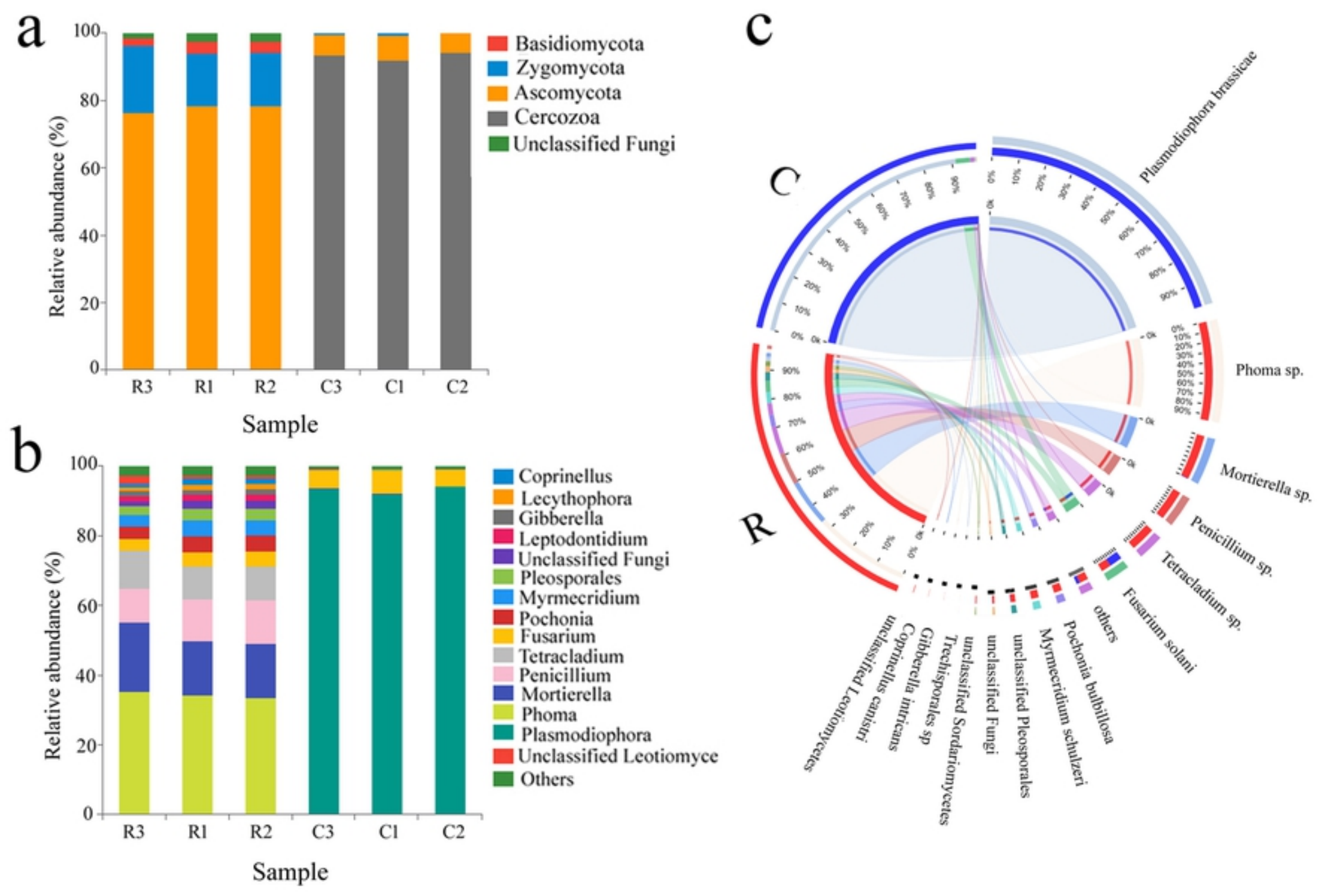
a
Relative abundance (%)
Sample
R3
R1
R2
C3
C1
C2
Basidiomycota
Zygomycota
Ascomycota
Cercozoa
Unclassified Fungi
b
Relative abundance (%)
Sample
Coprinellus
Lecythophora
Gibberella
Leptodontidium
Unclassified Fungi
Pleosporales
Myrmecridium
Pochonia
Fusarium
Tetracladium
Penicillium
Mortierella
Phoma
Plasmodiophora
Unclassified Leotiomyce
Others
c
C
R
Plasmodiophora brassicae
Phoma sp.
Mortierella sp.
Penicillium sp.
Tetracladium sp.
Fusarium solani
others
Pochonia bulbillosa
Myrmecridium schulzeri
unclassified Pleosporales
unclassified Fungi
unclassified Sordariomycetes
Trechisporales sp
Gibberella intricans
Coprinellus canistri
unclassified Leotiomycetes

Figure3

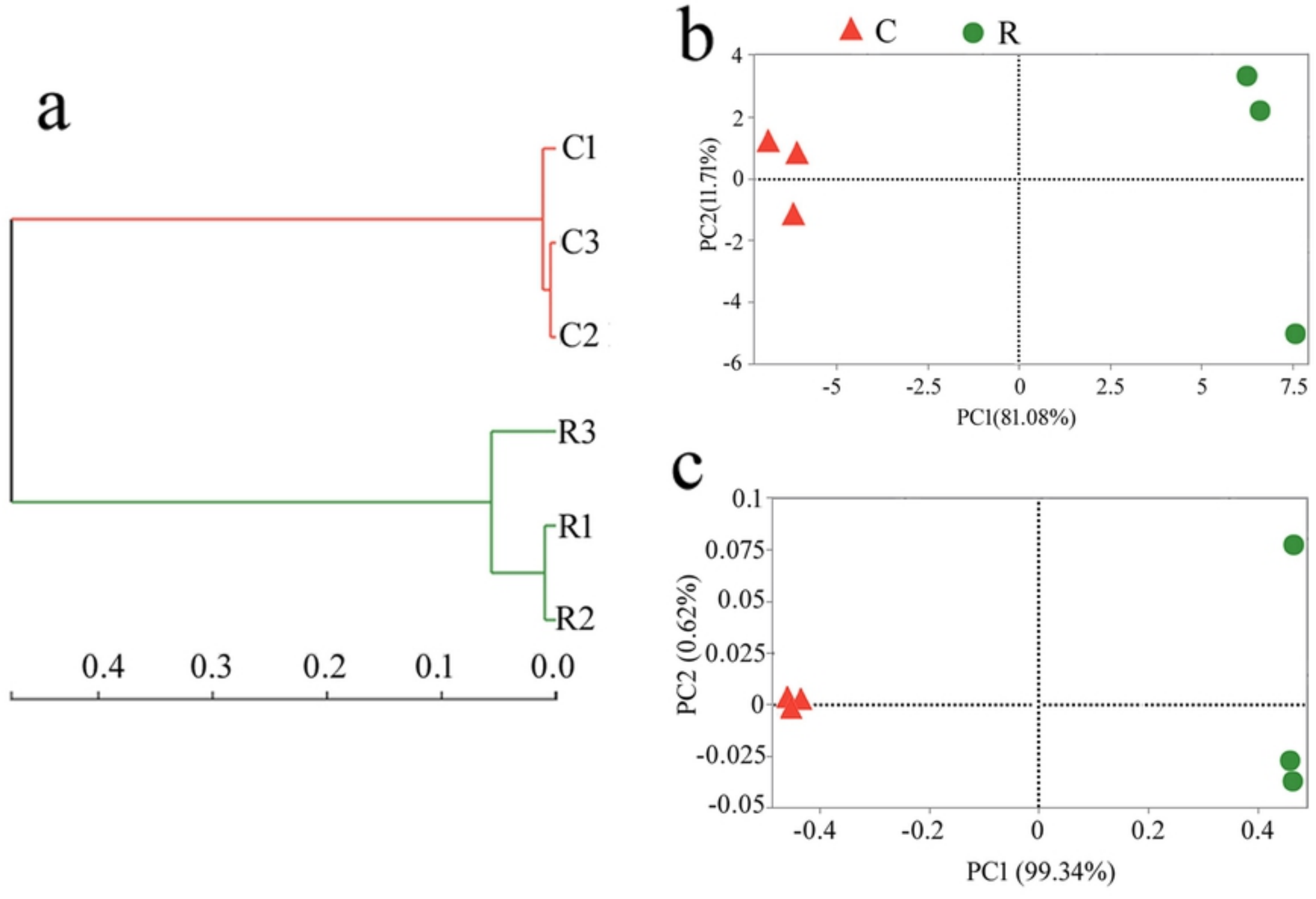
a
C1
C3
C2
R3
R1
R2
0.4
0.3
0.2
0.1
0.0
b
C
R
PC2(11.71%)
PC1(81.08%)
4
2
0
-2
-4
-6
-5
-2.5
0
2.5
5
7.5
c
PC2 (0.62%)
PC1 (99.34%)
0.1
0.075
0.05
0.025
0
-0.025
-0.05
-0.4
-0.2
0
0.2
0.4

Figure4

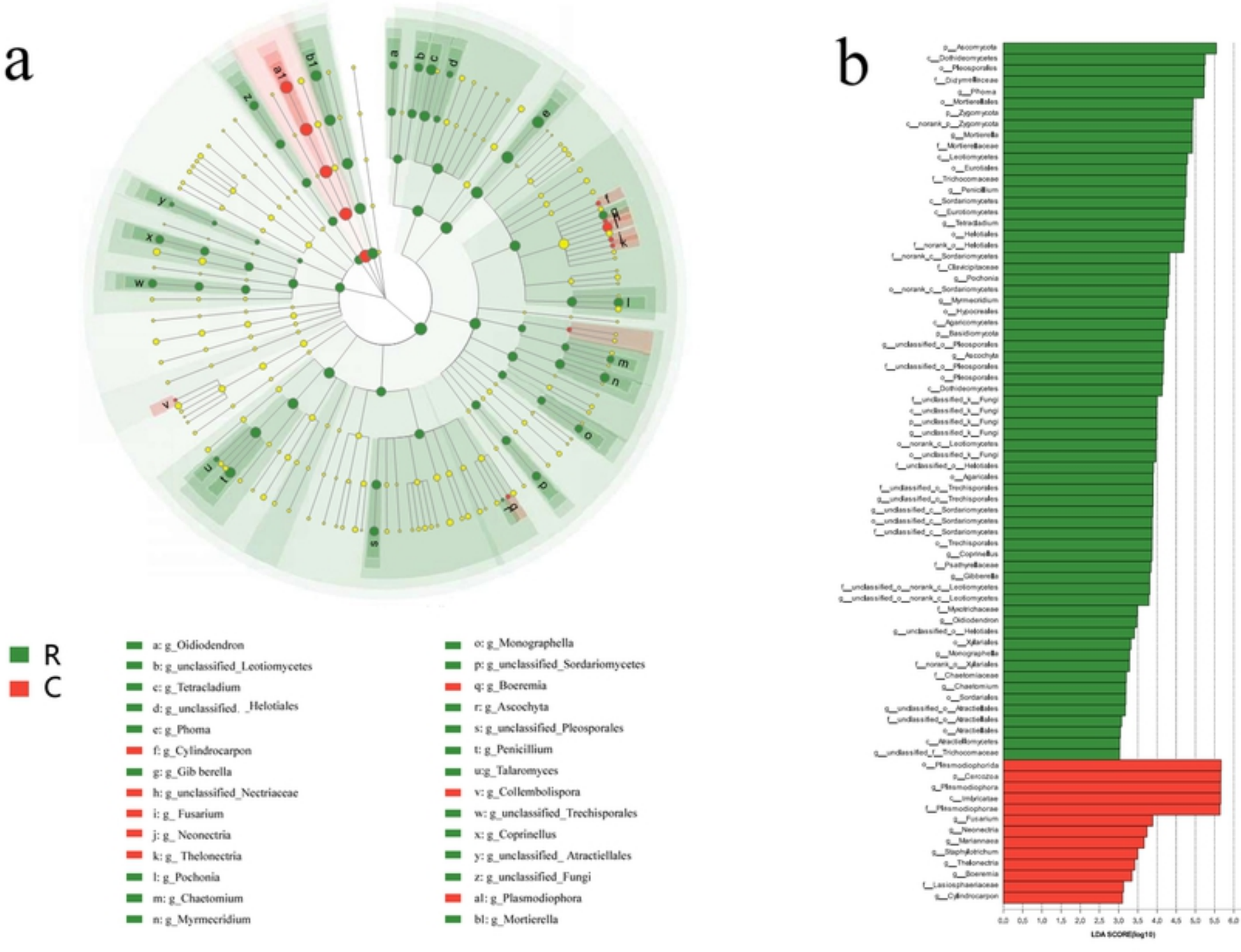
a
b
R
C
a: g_Oidiodendron
b: g_unclassified_Leotiomycetes
c: g_Tetracladium
d: g_unclassified_ _Helotiales
e: g_Phoma
f: g_Cylindrocarpon
g: g_Gib berella
h: g_unclassified_Nectriaceae
i: g_ Fusarium
j: g_ Neonectria
k: g_ Thelonectria
l: g_Pochonia
m: g_Chaetomium
n: g_Myrmecridium
o: g_Monographella
p: g_unclassified_Sordariomycetes
q: g_Boeremia
r: g_Ascochyta
s: g_unclassified_Pleosporales
t: g_Penicillium
u:g_Talaromyces
v: g_Collembolispora
w: g_unclassified_Trechisporales
x: g_Coprinellus
y: g_unclassified_ Atractiellales
z: g_unclassified_Fungi
a1: g_Plasmodiophora
b1: g_Mortierella
LDA SCORE(log10)

Figure5

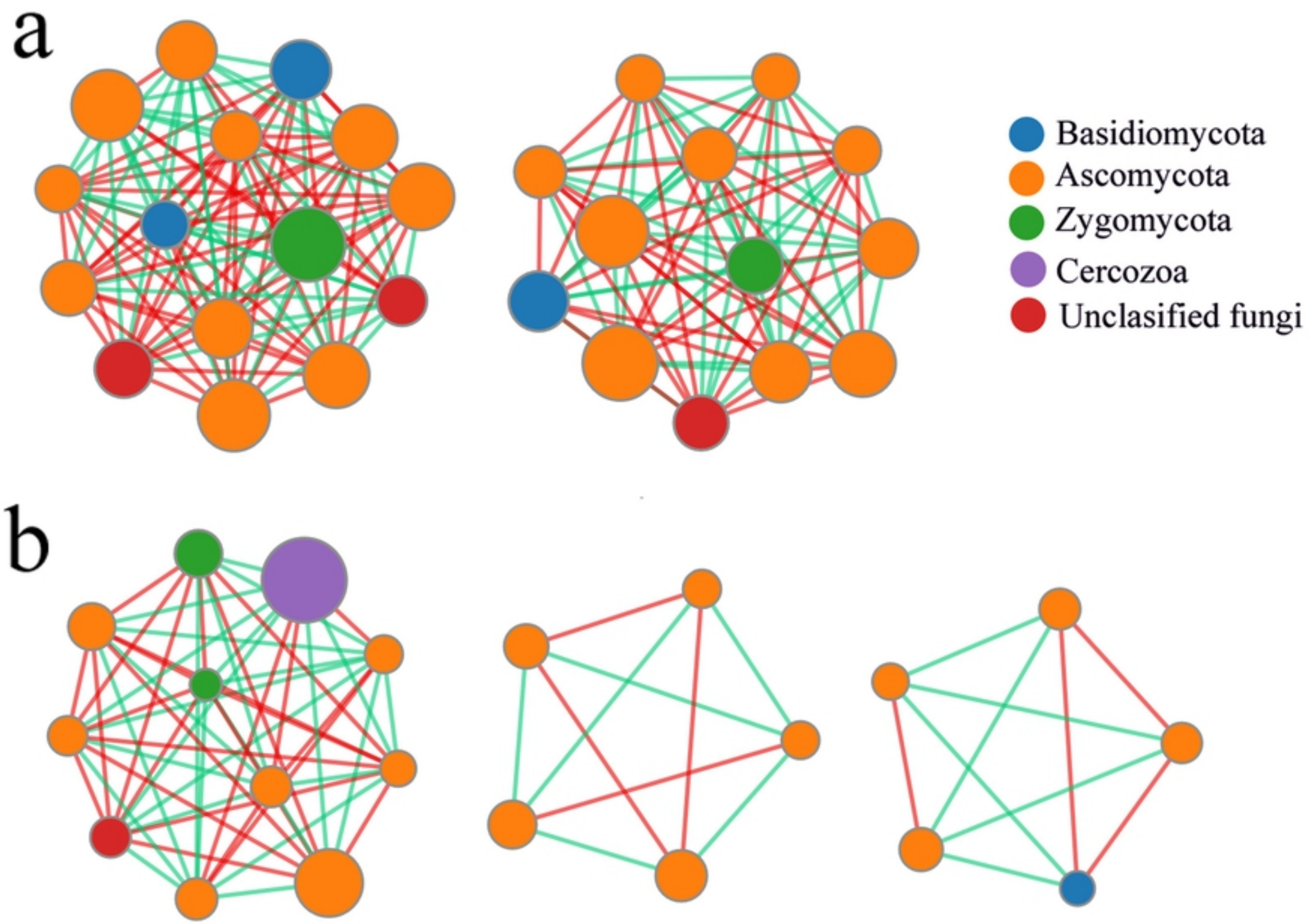
a
b
Basidiomycota
Ascomycota
Zygomycota
Cercozoa
Unclasified fungi

Figure6

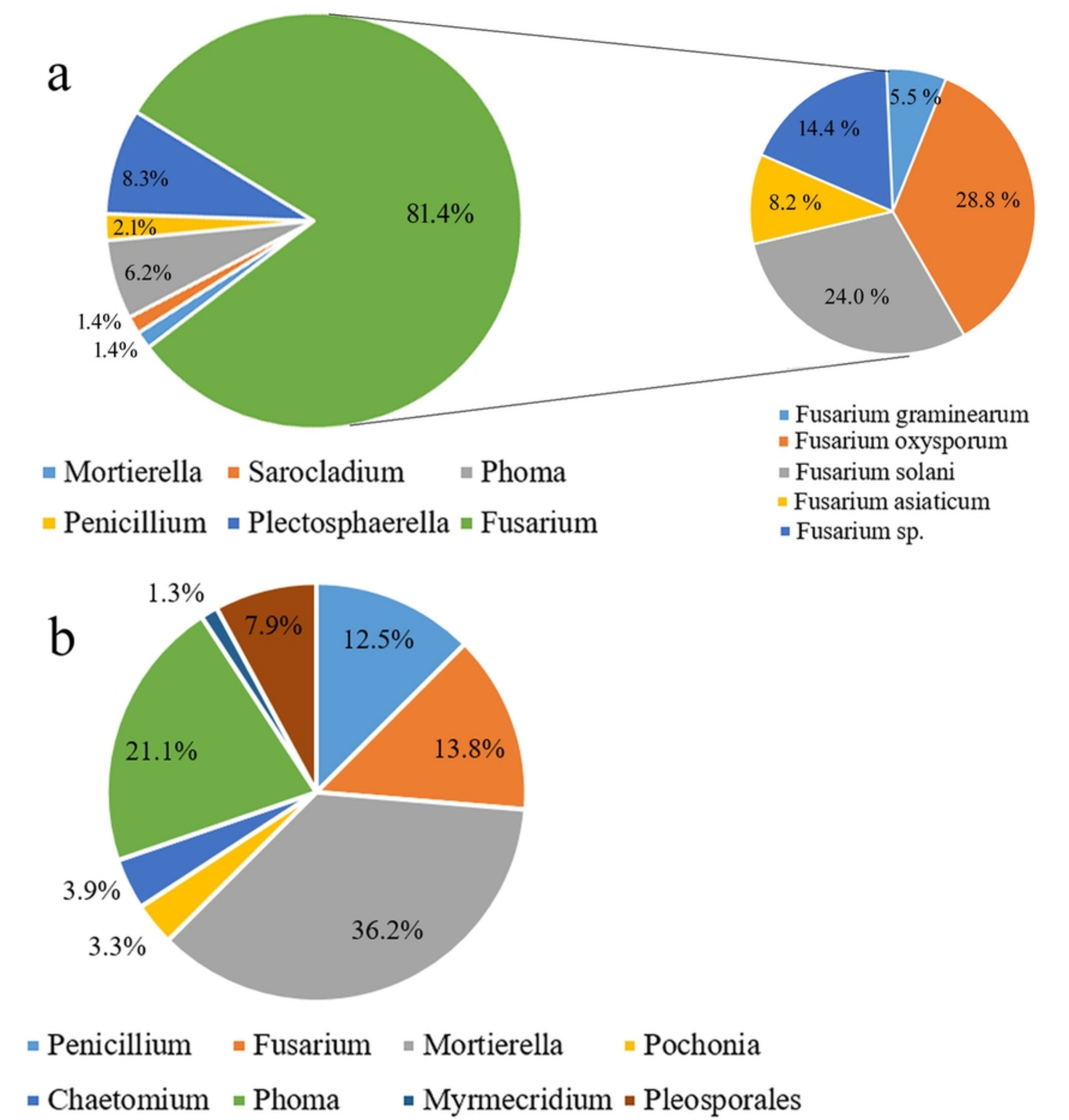
a
8.3%
2.1%
6.2%
1.4%
1.4%
81.4%
5.5 %
14.4 %
8.2 %
28.8 %
24.0 %
Mortierella
Sarocladium
Phoma
Penicillium
Plectosphaerella
Fusarium
Fusarium graminearum
Fusarium oxysporum
Fusarium solani
Fusarium asiaticum
Fusarium sp.
b
1.3%
7.9%
12.5%
21.1%
13.8%
3.9%
3.3%
36.2%
Penicillium
Fusarium
Mortierella
Pochonia
Chaetomium
Phoma
Myrmecridium
Pleosporales

Figure7