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Micro-community associated with ectomycorrhizal *Russula* symbiosis and sporocarp-producing *Russula* in Fagaceae dominant nature areas in southern China

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ABSTRACT *Russula griseocarnosa,* an ectomycorrhizal (ECM) fungus, is a species of precious wild edible mushrooms with very high market value in southern China. Its yield is affected by many factors including the tree species and environmental conditions such as soil microbiome, humidity. How the microbiome promotes the ECM fungus symbiosis with Fagaceae plants and sporocarp-producing has never been studied. In this study, we collected rhizosphere samples from Fujian province, the microbiota in the root and mycorrhizal rhizosphere were identified by Illumina MiSeq high-throughput sequencing. First, we compared three types of fungal communities: root tips infected with ECM *Russula* (type 1), tips with *Russula* sporocarp (type 2) and tips without ECM (type 3). Our results showed that the fungal richness was negatively correlated with *Russula*. *Russula*, *Tomentella* and *Lactarius* were common in Fagaceae ECM roots. As to the mycorrhizal interactions, *Boletu*s may be considered as an indicator species for sporocarp-producing *Russula*, and *Acremonium*, *Cladophialophora* were associated with *Russula* symbiosis. Second, we analyzed the fungal and bacterial

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communities in rhizosphere soils from the corresponding to previously three types (type 1, 2, 3). *Dacryobolus* and *Acidocella* may be considered as an indicator species for sporocarp-producing *Russula*. Fungi *Tomentella, Saitozyma*, *Elaphomyces* and bacteria *Acidicaldus*, *Bryobacter, Sorangium* and *Acidobacterium* occurred more frequently in the ECM *Russula* rhizosphere. Furthermore, the indicators *Elaphomyces*, *Tomentella, Sorangium* had a positive correlation with *Russula* symbiosis by network analyses. Overall, our results suggest a relationship between micro-community and ECM *Russula* formation and *Russula* sporocarp, which may provide new strategies for improving *Russula* symbiosis rate and sporocarp production.

KEYWORDS micro-Community, *Russula,* ectomycorrhizae, Fagaceae

IMPORTANCE *Russula* (Russulaceae, Russulales, Agaricomycetes, Basidiomycota) species are ectomycorrhizal (ECM) fungi that form symbiotic associations with host roots. Approximately 750 *Russula* species have been reported in worldwide (1). *Russula* taxa showed high diversity, strong habitat preference and some preference for soil horizons (2)(Jo´zsef Geml 2010). Li M(3)(2010)identified that *Russula* emerge at least three divergent lineages based on the genetic diversity and geographic differentiation in southern China , and *R. griseocarnosa* belongs to one of the lineages. *R. griseocarnosa* is described from southern China, including Guangdong, Yunnan, and Fujian Provinces (4). *R. griseocarnosa* sporocarp, which is popularly commercially as food and medicine, is uncultivatable and collected from the natural habitat. To date, there is little knowledge about the controlled production of *Russula* and its micro-community.

Several biotic factors affect ectomycorrhizal fungal communities: plant host species (5, 6) , plantation age (7), asymptomatic ectomycorrhiza endophytes (8), ectomycorrhizal propagules in the soil(9) and the traits of the dispersal propagules of ECM fungi (10). The common ectomycorrhizal *Russula* sp. was associated with seven host genera: *Pleioblastus chino*, *Quercus serrata*, *Symplocos prunifolia*, *Ilex pedunculosa*, *Prunus jamasakura*, *Gamblea innovans*, and *Lyonia ovalifolia*(11, 12). *R. griseocarnosa* grows in forests with Fagaceae in southern China (13). ECM

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fungal communities strongly vary during long-term ecosystem development, even within the same hosts (14). Some *Russula* spp. was reported to be dominant colonizers of mature roots of pines (15)(Horton & Bruns, 2001) but did not colonize bioassay seedlings (16).'Early stage' and 'late stage' mycorrhizal fungi of birch appeared distinction (7). Gebhardt also found that Ectomycorrhiza communities of *Quercus rubra* of different age performed specificity with low similarity between the chronosequence stands site (17) . Some fungal root endophytes prefer ECM formed by particular species of ectomycorrhizal fungi (8, 18), potentially representing a second level of root endophyte selection. Dark septate endophytes are considered to be ubiquitous, colonizing mycorrhizal(19). ECM propagule communities in soil may diverge from those root-colonizing ECM communities and affect persistence of symbiotic relationship between mycorrhiza and available host roots by competitive networks(20). ECM propagules in soil are less frequent and diverse in early primary succession and become more frequent and diverse along forest development, due mainly to the accumulation of dormant spores of *Rhizopogon* spp. and sclerotia of *Cenococcum* spp(20). Simultaneously dispersal ability across ECM species correlated well with the composition of communities associated with host (21). *Russula* naturally propagate by short-distance spore dispersal rather than vegetative growth of dikaryophytic mycelia or long-distance spore dispersal(22). In all, the *Russula* ECM community may be affected by symbiotic structures and root endophytes in the roots, mycorrhizal extraradical mycelia, spores in the soil , as well as its dispersal ability.

Mycorrhizal interactions are usually classified on the basis of the features of the symbiotic interfaces(23). The ECM community colonizing root tips was strongly structured by competitive interactions or ecological processes generating a similar spatial pattern, rather than neutral processes. The ECM *Cortinarius* sp. and *Lactarius rufus* competed for root tips(24). Both *Cenococcum geophilum* and *Clavulina cinerea* as mycorrhizas and as extramatrical mycelium (EMM) in a *Pinus resinosa* plantation showed a negative correlation(25). Hortal suggests that

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interspecific competition between *Lactarius deliciosus* (inoculated fungi) and *Rhizopogon roseolus* (indigenous fungi) occurs in the root system for ectomycorrhiza formation in available roots, rather than in the extramatrical mycelium phase (26). However, associations among *Suillus bovinus* and *Gomphidius roseus* occur within ectomycorrhizal roots (27). There are also a number of notable examples of associations between sporocarps of different species, such as between *Boletus parasiticus* and *S. citrinum*, *Asterophora parasitica* and *Lactarius*, and between *Claudopus parasiticus* and *Cantharellus cibarius* (28),. However, mycorrhizal interaction studies of *Russula* are limited in the literature.

Similar to the rhizosphere, mycorrhizosphere is the feet of fruiting bodies or the root of ectomycorrhizal fungi . Mycorrhizosphere may constitute a hot spot(29), in which microorganisms were affected(30-32).The mycosphere effect was prominent , though previous studies have failed to demonstrate significant differences between bacterial communities associated with roots colonized by *Suillus variegatus* and *T. submollis*, *Lactarius* sp. and *Tomentella* sp.(33) or by *C. geophilum* and *Russula* sp. (34). It has also been established that mycorrhizal networks have different microbial communities compared with bulk soil by stimulating some families such as *Bradyrhizobiaceae*, *Burkholderiaceae*, and *Pseudomonadaceae* (35). Bacterial activity is stimulated by the provision of easily available nutrition such as carbonaceous compounds (30).The mycosphere effect might exert strong influences on the bacterial community of soil(36)**,** in which bacterial activity is stimulated by trehalose, which is degraded by hyphosphere and derived from fungi (37, 38). Andersson(39)(2003)., who used phospholipid fatty acid profiles to characterize bacterial communities, has demonstrated that basidiomycete wood-decomposing fungi are able to influence bacterial community structure. Bacteria adapted to the mycospheres of three or more or just one fungal species was defined as specific selective bacterial(40). The specific members of the Sphingomonadaceae family selected were at the bases of the fruiting bodies of the ectomycorrhizal fungi *Laccaria proxima* and *Russula exalbicans* in comparison to the adjacent bulk soil,

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major of which did not cluster with known bacteria from the database (36, 41). On the other hand, some mycorrhiza-associated bacteria have been shown to produce compounds that are antagonistic to plant pathogens (42). Meanwhile, "mycorrhiza helper bacteria" (MHB) appeared to positively influence the development and function of ectomycorrhiza (43), but the effects varied with different species combinations (44, 45). *Ralstonia* sp. and *Bacillus subtilis* can promote *Suillus granulatus*-infected *Pinus thunbergii* (34). *Bacillus subtilis* helped the growth of *Cenococcum geophilum* Fr and promoted *Suillus granulatus* infection (34) but inhibited *Rhizopogon* sp. infection (45). Apart from bacterial , ectomycorrhizal symbionts strong also selected ascomycete communities and other ECMs(31) (32).Högberg (2002)(46) demonstrated that the EMM is at least 30% of the microbial biomass in boreal forest soils. ECM fungi competed with saprotrophic fungi in soil by the EMM(47). *Tuber rufum* and some members of *Boletales* are typically restricted to productive truffle plots. On the other hand, *Hebeloma*, *Laccaria* and *Russula* species are mostly associated with unproductive truffle plots, Ectomycorrhizae belonging to *Thelephoraceae* are frequently found in mature truffle orchards but do not seem to affect sporocarp production (32). Ascomycetes associated with ectomycorrhizas: molecular diversity and ecology with particular reference to the Helotiales had been reported (48).However, the rhizosphere, mycosphere effect of *Russula griseocarnosa* has not been recorded in the literature.

Regarding the relationship between the amount of soil EMM with ectomycorrhizae and sporocarps, different mycorrhizas have different conclusions . Zampieri et al.(2010)(49) showed that the mycelium of *Tuber magnatum* was more widespread than was inferred from the distribution of its fruiting bodies and ectomycorrhizae. Zhou et al. (2001) (50)demonstrated that the development of *Suillus grevillei* sporocarps is correlated with amount of EMM and ectomycorrhizae of *S. grevillei* in a narrow area. The *Tuber melanosporum* EMM biomass detected in the soil from the natural truffle ground was significantly greater than that of other plant orchards analyzed, and the lowest amount of *T. melanosporum* mycelium maintained a

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sporocarp production in plant orchards (51). However, Some ECMs are consistent in ectomycorrhizae but inconsistent in sporocarps. De la Varga et al. (52)(2012) quantified *B. edulis* extraradical mycelium in a Scots pine forest and found a positive correlation between the amount of mycelia and the presence of *Boletus edulis* mycorrhizae, but not with the productivity of fruiting bodies. However, the relationship between the amount of *Russula* with ectomycorrhizae and sporocarps is scarce in the literature.

The relation between the productivity of fruiting bodies and ECM symbiosis is still unclear. Some ectomycorrhizal species produce abundant ectomycorrhizal root tips but few or no fruiting bodies, while other ectomycorrhizal fungi form abundant fruiting bodies but a low number of ectomycorrhizal root tips. Guidot et al. (53)(2001) found a spatial congruence of above- and belowground distribution for *H. cylindrosporum* . However, De la Varga et al.(52) (2012) found that the presence of mycorrhizae of the *B. edulis* symbiotic rate was not consistent with the production of fruiting bodies. There are also *Russula* species difference between the above-ground *Russula* sporocarp and underground *Russula* mycorrhizal(2) . Geml(2010) (2)observed that 15 and 45 of the 50 *Russula* phylogroups species were found in sporocarp and soil clone sequences, respectively. Given the long delay between the establishment of the plantation and the formation of sporocarps, short- and medium-term control of the survival and persistence of fungal symbionts in plantations have to still be evaluated by the assessment of vegetative structures as the ectomycorrhizas or extraradical mycelium.

Microbial community affected mycorrhizal fungal function (for example, symbiosis establishment capacity, sporocarp production), and the reciprocal effects are vice versa. The microorganisms associated with mycorrhizal fungi may either have positive or negative impacts on fungal spore germination, growth, nutrient acquisition and plant colonization (43, 54). *Tuber indicum* altered the ectomycorrhizosphere and endoectomycosphere microbiome and metabolic profiles of the host tree *Quercus aliena* (55). *Tuber borchii* shapes the ectomycorrhizosphere microbial communities of *Corylus avellana*(56). Therefore,

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we speculate that detecting the communities of *Russula* can tracked ECM persistence throughout the entire biological cycle, which will help to control ectomycorrhiza formation and sporocarp production. In this study, to understand the communities of the targeted *Russula griseocarnosa* and to find the possible indicator microbes of successful *Russula griseocarnosa* plantations, we identified the *Russula* ectomycorrhizal fungal communities inhabiting different life cycle stages based on MiSeq sequencing of ribosomal internal transcribed spacer (ITS) sequences of root DNA and mycosphere communities based on MiSeq sequencing of the 16S V3-V4 as well as ribosomal internal transcribed spacer (ITS) sequences of mycosphere soil DNA.

This study is the first attempt to analyze ectomycorrhizal communities of *Russula* using MiSeq sequencing metagenomics DNA of *Russula* root and the Mycorrhizal rhizosphere soil in *Russula* at different stages. We think that *Russula* shapes the ectomycorrhizosphere microbial communities of Fagaceae *(Quercus glauca* and *Castanopsis hainanensis*.).

RESULTS

Comparing microbiomes among types of the ECM fungus Russula. ECM *Russula* was first identified by combining morph typing with Sanger sequencing DNA sequences . We analyzed internal transcribed spacer (ITS) rDNA sequences of ECM root tips and ECM rhizosphere soil samples using phylogenetic methods, operational taxonomic unit (OTU) delimitations and ordinations to compare species composition in various types of ECM *Russula*.

In the ECM metagenome, we found a positive correlation between the concentration of *Russula* DNA and the presence of *Russula* mycorrhizae in the mycorrhizal rhizosphere and ECM root (Tab 1). To analyze whether distinctive communities are selected by *Russula* ectomycorrhizal fungi, the fungi of ECM root microbiomes were compared and divided into three types. *Russula* that could be detected by Sanger sequencing with a DNA concentration above 10% of total ECM

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genomic DNA, as determined by MiSeq sequencing, were classified as type 1. *Russula* sporocarps that could be collected from the ground and the extended hyphae of which could be connected between roots of the host and sporocarp within 50 cm, with *Russula* detected by Sanger sequencing, were classified as type 2. *Russula* that were not detected by Sanger sequencing or with a DNA concentration below 5% of the total ECM genomic DNA, as determined by MiSeq sequencing, were classified as type 3. Therefore, the samples of ECM tip and rhizosphere samplings were divided into three types: *Russula*-infected (type 1), sporocarp-producing Russula (type 2), and Russula-uninfected (type 3) (Tab 1). Relative *Russula* OUT abundance is significant difference in ECM *Russula* symbiosis root and in ECM *Russula* rhizosphere, respectively (Tab 1, Fig. 1A).Type 2 is the most abundant, type 1 is the second, and type 3 is the least. Interestingly, in type 3, the amount of *Russula* in the soil is higher than in the root. This may indicate that in the natural growth area of *Russula*, there are a large number of *Russula* propagules in the soil.

TABLE 1 Relative *Russula* OUT abundance in three types and Alpha diversity analysis of fungi and bacterial of three types of ECM *Russula* roots or *Russula* rhizosphere soil in natural *Russula* growth areas

Type	Relative Russula OUT abundance (%) in ECM	Chao1richness of Fungi Community of ECM Russula root1	Shannon of Fungi Community of ECM Russula root	Relative Russula OUT abundance (%) in ECM	Chao 1 richness of Fungi Community of ECM Russu	Shannon of Fungi Community of ECM Russula rhizos	Chao 1 richness of Bacterial Community of ECM	Shannon of Bacterial Community of ECM Russula

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	Russula root			Russula rhizosphere			Russula rhizosphere	Rhizosphere
Russula–infected (type1)	28.74 ± 19.31 B[1,2]	663.12± 171.655 b	4.04± 1.007 b	20.22 ± 4.59B	901.38± 32.30 a	4.52± 0.53a	1010.28± 17.29 b	7.54± 0.10b
Russula sporocarp producing (type2)	66.66 ± 12.92 C	170.44± 24.68c	2.20± 0.19 c	39.23 ± 7.58C	756.87± 52.18 b	4.56± 0.99a	945.81± 25.08 b	7.49± 0.049 b
Russula-no-infected (type3)	1.44± 1.02 A	782.20± 102.345 a	4.55± 0.921 a	5.61± 1.71A	874.19± 41.16 a	4.47± 0.95a	1099.52± 13.14 a	7.94± 0.13a

1. mean±standard deviation ; type1has 4 repeats, type2 has 2 repeats and type3 has 3 repeats.

2. Analysis of variance by one-way analysis of variance In each column: different words indicate differences, uppercase letters indicate extremely significant differences ($P < 0.01$); and lowercase letters indicate significant differences ($P < 0.05$)

Fungal diversity analysis and analysis of indicator species associated with ECM *Russula* roots. In total, 1346 fungal operational taxonomic units (OTUs) were distinguished in roots. Among the three types, the Chao1 diversity index and Shannon diversity index decreased in sporocarp-producing *Russula* type (type 2)

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and *Russula*-infected type (type 1) compared to the *Russula*-uninfected (type 3) (Tab 1). The result shows that species richness shifted in the composition of the ECM community associated with *Russula*.The fungal community composition and the abundance of the main fungi (over 0.05% fungi in roots genome) were different in the three *Russula* types roots (Fig. 1A,Tab 1).The result shows that *Russula* is dominates in fruiting bodies (type 2) and infected samples(type 1) though *Russula* in three type samples.

Based on the top 20 fungi of each sample, we first obtained the common species of three types after intersecting each type with the Venny mapping tool: 14 species were in type 2, 5 in type 2 and 3 in type 3 (Tab S1). Then, we intersected the common types of three types with the Venny mapping tool. At the genus level, the three types shared 3 common genera: *Russula*, *Tomentella* and *Lactarius* (Tab S2, Fig. 1B).These results showed that these are common fungi in Fagaceae ECM roots. Types 1 and 2 shared 2 common genera: *Acremonium* and *Cladophialophora* , in addition to the abovementioned three common ECM(Tab S2,Fig. 1B). Second, We take a collection of three types genera respectively, subsequently we intersected the collection of three types by the Venny mapping tool (Fig. 1C): 24 species in type 2, 46 in type 1 and 41 in type 3, and 5 genera were exclusively in type 2 collection. We take the intersection of the unique collection (9 genera) and union (5 genera) in type 2, and found only one species boletus (Fig. 1D , Tab. 2). Analyze fungi composition differences of three types roots by PCoA based on the top 20 fungal genera , we found that the control (type 3) belonged to quadrant IV, type 1 belonged to quadrant I or II, and type 2 belonged to quadrant III (Fig. 1F). The results show that the fungal community of sporocarp- producing *Russula* was completely different from that of Russula-uninfected . The *Russula*-infected type was in the transitional phase.We suggest the *Russula* infection contributed 31.3% and others elements host contributed 20.5% of the differences, respectively. *Boletus* was the only ECM with the emergence of *Russula* fruit bodies. In all, *Boletus* may be considered an indicator species in the *Russula* sporocarp-producing fungal community, and *Acremonium* and *Cladophialophora* may be considered indicator

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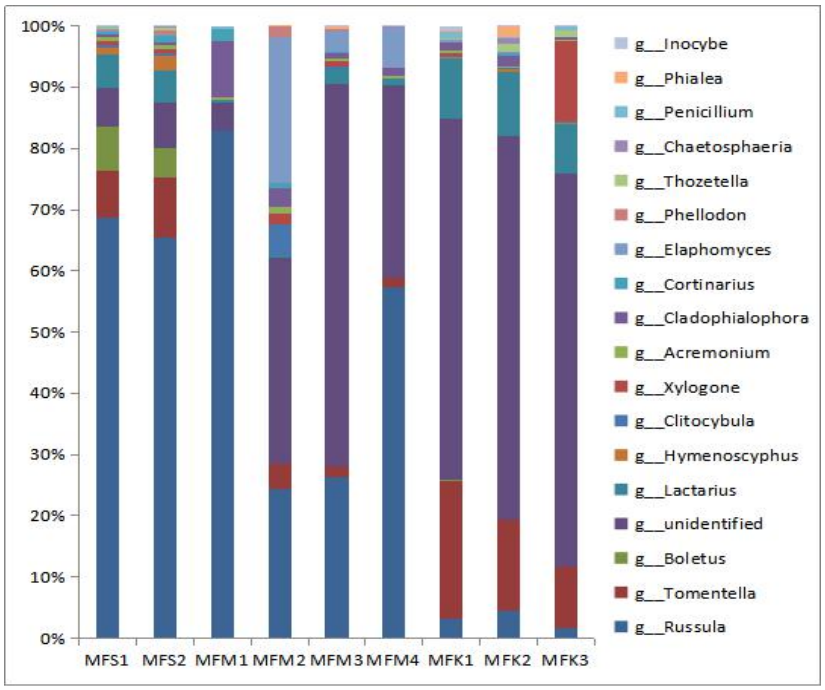
species in *Russula* symbiosis fungal communities (Tab. 2). Analyze the different effects of ECM species by PCoA based on the top 20 fungal genera, we found *Tomentella* ,*Xylogone* ,and *Lactarius* belonged to I, II, while others belonged to quadrant III,V (Fig. 1E). The *Russula* infection contributed 55% and others elements contributed 24% of the differences, respectively. Combining these two factors, it shows *Russula* and *Elaphomyces* can be divided into one categories, while *Xylogone* can be divided into another category functionally. So we assume that *Elaphomyces* are benefit for *Russula* symbiosis while *Tomentella* ,*Xylogone* ,and *Lactarius* has the function of competing hosts.

A



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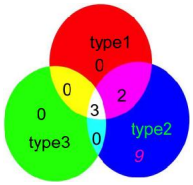

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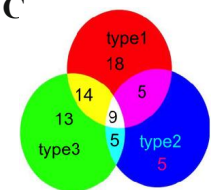
C



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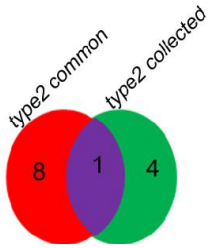

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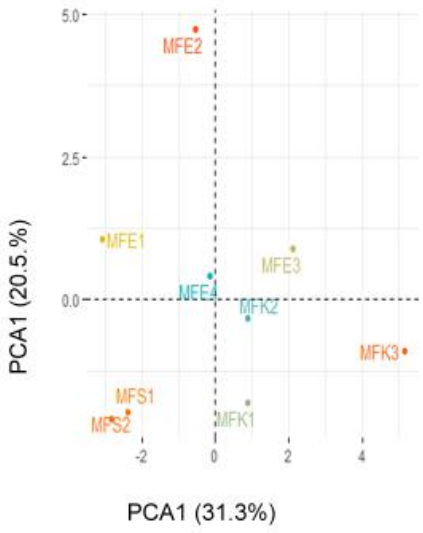

Fig. 1 Diversity analysis of fungi of three types of *Russula* symbiosis roots in natural Russula growth areas

A:The main fungi genus composition and abundance in type2 *Russula* symbiosis root and the corresponding genus are in the other two types

B: The Venny mapping of the common genus of the top 20 fungi genus in three types Russula symbiosis root

C: The Venny mapping of the collection genus of the top 20 fungi genus in three

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329 types Russula symbiosis root

330 D: The Venny mapping of the common genus and the collection genus of the top 20

331 fungi genus in type2 Russula symbiosis root

332 E : Analysis of ECM role difference of three types Russula symbiosis root on top 20

333 fungi genus of ECM Russula by PCoA

334 F :Analysis of composition difference of three types Russula symbiosis root on top

335 20 fungi genus of ECM Russula by PCoA

336 In Eand F: The scales of the horizontal and vertical axes are relative distances and

337 have no practical significance. The contribution rate is the degree of interpretation,

338 and the hypothetical factors can be evaluated and verified.

339 In A,Eand F :In the sample name, the first letter M indicates mycorrhizal root ; first

340 second F stands for fungi; the third letter different treatment :S indicates

341 sporocarp(type2), E indicates ECM ectomycorrhizal(type1) , K indicates

342 control(type3) respectively; the fourth number indicates the different biological

343 repetitions of different treatment .

344

345 **Fungal diversity analysis and analysis of indicator species associated with**

346 **ECM *Russula* rhizosphere soil.** In total, 1829 fungal operational taxonomic units

347 (OTUs) were distinguished in rhizosphere soil. Interactions with native

348 ectomycorrhizal fungi present in the soil play a key role in the higher diversity of

349 fungal taxa .Compared to type 1 and 3, the Chao1 diversity index decreased in

350 types 2 ; while there were no significant differences between types 1 and 3 (Tab. 1).

351 But, the Shannon diversity index showed no significant differences in three types

352 (Tab. 1). The fungal community composition and

353 A

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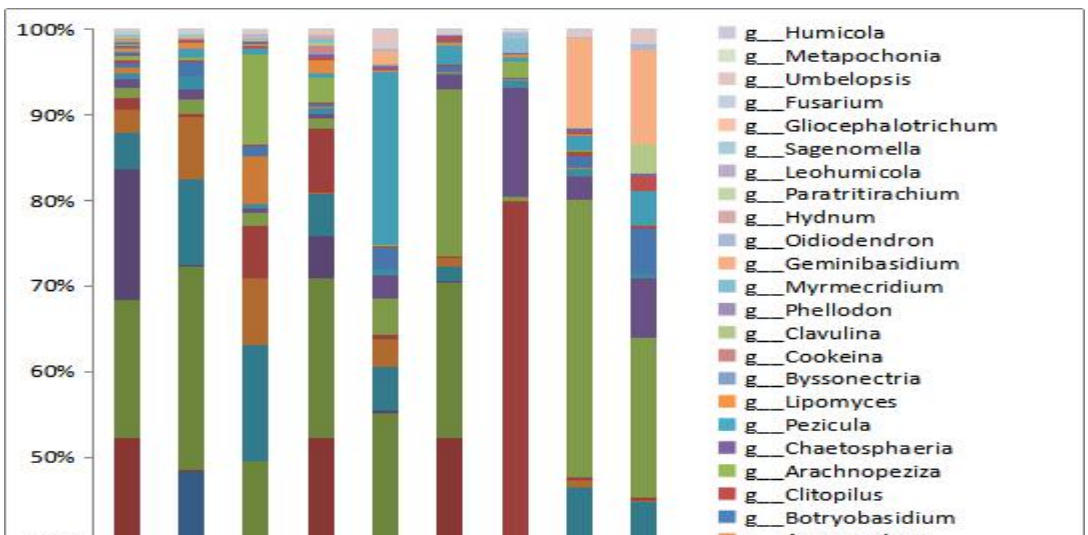

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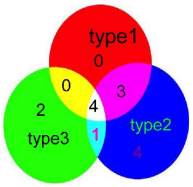

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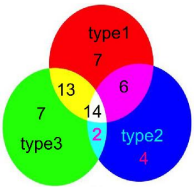

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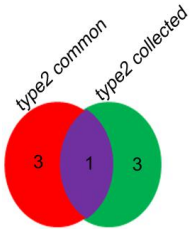

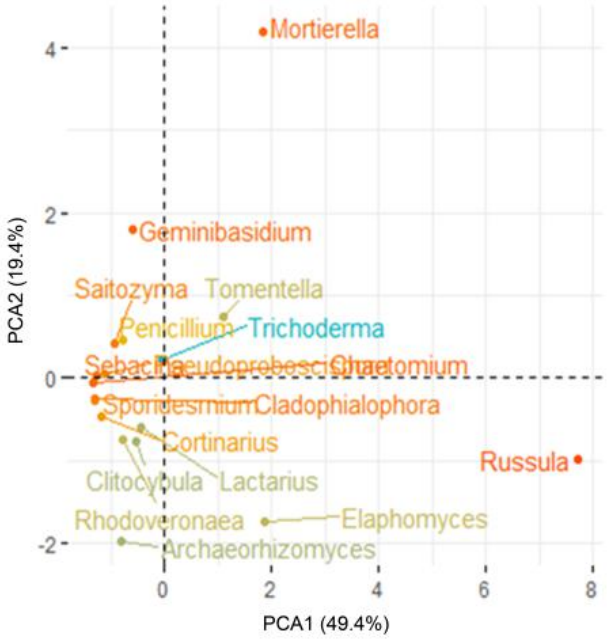

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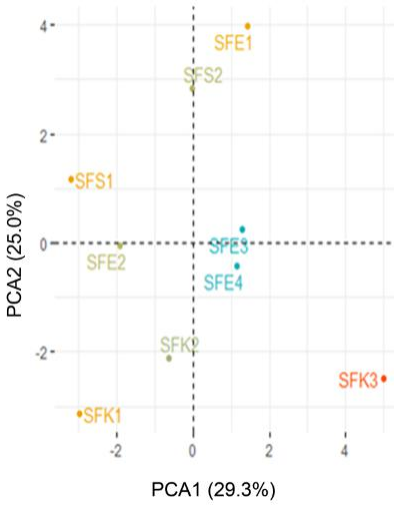

Fig. 2 Diversity analysis of fungi of three types *Russula* mycorrhizal rhizosphere in natural *Russula* growth areas

A:The top 20 fungi genus **c**omposition and abundance in type2 *Russula* symbiosis mycorrhizal rhizosphere and the corresponding genus are in the other two types in at Fujian,China

In the sample name, the first letter S indicates mycorrhizal root soil ; first second F stands for fungi; the third letter different treatment :S indicates sporocarp, E indicates ECM ectomycorrhizal , K indicates control respectively; the fourth number indicates the different biological repetitions of different treatment .

B: The Venny mapping of the common genus of the top 20 fungi genus in three types *Russula* symbiosis Mycorrhizal rhizosphere

C: The Venny mapping of the collection genus of the top 20 fungi genus in three types *Russula* symbiosis Mycorrhizal rhizosphere

D: The Venny mapping of the common genus and the collection genus of the top 20 fungi genus in type2 *Russula* symbiosis Mycorrhizal rhizosphere

E : Analysis of ECM role difference of three types Russula symbiosis mycorrhizal rhizosphere on top 20 fungi genus of ECM Russula by PCoA

F :Analysis of composition difference of three types Russula symbiosis mycorrhizal rhizosphere on top 20 fungi genus of ECM Russula by PCoA

In E and F: the scales of the horizontal and vertical axes are relative distances and have no practical significance. The contribution rate is the degree of interpretation,

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and the hypothetical factors can be evaluated and verified.

the abundance of the main fungi (over 0.05% fungi in soil genome)were different in the three *Russula* types rhizosphere soil (Fig. 1A,Tab 1).Compared to the root ,The result shows that *Russula* is also dominates in fruiting bodies (type 2) and infected samples(type 1) though *Russula* in three type samples(Fig. 1A,Fig. 2A). However,compared to the root ,the genera variety increased (Fig. 1A,Fig. 2A).

The community composition of the top 20 fungi in the three *Russula* rhizosphere soil types was analyzed (Tab. S2). We first obtained the common species of three types after intersecting each type using the Venny mapping tool: 12 species in type 2, 7 species in type 1 and 7 species in type 3 (Fig. 2B). Then, we intersected the common types of the three types with the Venny mapping tool. At the genus level, the three types shared 4 common genera: *Russula, Mortierella*, *Penicillium* and *Trichoderma*; and *Russula* also had a large frequency in types 1 and 2 (Fig. 2B). This showed that 4 genera are common fungi in Fagaceae-dominant rhizosphere soil. Types 1 and 2 shared 3 common genera: *Elaphomyces*, *Tomentella* and *Saitozyma* ,in addition to the abovementioned 4 common genera(Fig. 2B). Second, we take a collection of three types genera respectively, subsequently intersected the collection of three types by the Venny mapping tool (Fig. 2C): 26 species in type 2, 40 in type 1 and 36 in type 3; 4 genera were included exclusively in the type 2 collection. We take the intersection of the unique collection (4 genera) and union (4 genera) in type 2, and found *Dacryobolus* unique belonged to type 2 (Fig. 2D, Tab. 2). The top 20 fungal genera in the sporocarp-producing *Russula* root rhizosphere with types 3 or types 1 were compared. The result shows that *Russula* EMM is dominates in fruiting bodies (type 2) and infected samples (type 1) though *Russula* in three type samples. *Elaphomyces* also dominated in types 1 and 2, but nearly did not exist in the control, type 3 (Fig. 2D). In all, *Dacryobolus* may be considered an indicator species of sporocarp-producing *Russula* in the *Russula* rhizosphere. *Elaphomyces*, and*Tomentella* may be considered indicator species for *Russula* symbiosis in the rhizosphere (Tab. 2). Analyze three types of fungi composition differences of top 20 genera by PCoA in *Russula* rhizosphere soil based on the top

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449 20 fungal genera, we found that type 2 belonged to quadrant II and type 1 belonged
450 to quadrants I, III and IV, while type 3 belonged to quadrants III and IV, down the
451 horizontal axis (Fig. 2E). The host and *Russula*-infection contributed 29.3% and
452 25.0%, respectively. Furthermore ,analyzing the different effects of fungi species by
453 PCoA , we found *Russula* and *Elaphomyces* belonged to quadrant IV, *Mortierella*
454 and *Tomentella* belonged to quadrant I, *Trichoderma*, *Penicillium* , *Geminibasidium*
455 and *Saitozyma* belonged to quadrant II, while others belonged to quadrant III (Fig.
456 2E). The *Russula* infection contributed 49.4% and others elements host contributed
457 19.4% of the differences, respectively. Combining these two factors, we assume
458 that *Elaphomyces* in soil are benefit for *Russula* symbiosis.
459
460 **Bacterial diversity analysis and analysis of indicator species associated with**
461 **ECM *Russula* rhizosphere soil.** In total, 1494 bacterial operational taxonomic
462 units (OTUs) were distinguished in this study. Compared to type 3, the Chao1
463 diversity index decreased in types 1 and 2 (Tab. 1). There were significant
464 differences between type 3 and the other two types. However, there were no
465 significant differences between types 1 and 2. The Shannon diversity index showed
466 the same tendency as the Chao1 diversity index (Tab. 1). The bacterial community
467 composition and the abundance of the main bacterial (the over 0.05% bacteria in
468 soil genome) were analyzed (Fig. 3A). About 40-60% of the species in the sample
469 cannot be identified by Illumina MiSeq high-throughput sequencing and the
470 remaining bacterial genera in the sporocarp-producing *Russula* root rhizosphere soil
471 were showed for the three types (Fig. 3A). Compared to the fungi of the root and
472 rhizosphere soil, the bacterial genera variety still increased (Fig. 1A, 2A, 3A).
473 The community composition of the top 20 f bacteria in the three *Russula*
474 rhizosphere soil types was analyzed (Tab. S3).We first obtained the common
475 species of the three types after intersecting each type with the Venny mapping tool:
476 18 species in type 2, 14 in type 1 and 12 in type 3 (Fig. 3B). Then, we intersected
477 the common types of the three types with the Venny mapping tool. At the genus
478 level, the three types shared 10 common genera: *Acidibacter*, *Candidatus*,

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Rhizomicrobium, Acidothermus, Variibacter, Burkholderia, Roseiarcus, Bradyrhizobium, Candidatus and *Granulicella*. (Fig. 3B). This showed that 10 genera are common bacteria in Fagaceae-dominant rhizosphere soil. Types 1 and 2 shared 4 common genera: *Bryobacter*, *Sorangium, Acidicaldus*, and *Acidobacterium* ,,in addition to the abovementioned 10 common genera(Fig. 3B). Second, we obtained the collection of the various types: 20 species in type 2, 27 in type 1 and 26 in type 3 (Fig. 3C,Tab S3). Then, the unique genera were analyzed of the unique collection (4 genera) and union (3 genera) in type 2 by the Venny mapping tool, and 2 genera ***Acidocella*** and ***Edaphobacter*** were found (Fig. 3D). In all, *Acidocella* ,*Edaphobacter* may be considered indicator species for sporocarp-producing *Russula*, and *Bryobacter*, *Sorangium, Acidicaldus*, and *Acidobacterium* can be considered indicator species for *Russula* symbiosis in the bacterial community (Tab. 2). By PCoA based on the top 20 fungal genera, we found that type 2 belonged to quadrant III , left of the vertical axis. Except for one sample, other samples of type 1 belonged to quadrants II or III, left of the vertical axis . Type 3 belonged to the right of the vertical axis (Fig. 3F). We assume that the *Russula* infection and host contributed 47.3% and 21.4%, respectively(Fig. 3F). Analyze the different effects of bacteria species by PCoA , we found ***Acidocella***, *Sorangium*, *Haliangium* , *Telmatospirillum* , ***Edaphobacter*** , *Acidobacterium* , *Sphingomonas*, *Candidatus_Koribacter* , *Roseiarcus* , *Granulicella* and *Acidicaldus* belonged to quadrant II, III , while the others belonged to quadrant I, IV (Fig. 3E). It shows 11 gengus in quadrant II, III can be divided into one categories by role. All indicator species for sporocarp-producing *Russula* or *Russula* symbiosis in the same categories but *Bryobacter*. We assume that the *Russula* infection contributed 80.9% and others elements contributed 10.5% respectively. Combined with indicator species analysis, we assume that 11 bacterial gengus beneficial to the symbiosis of *Russula*.

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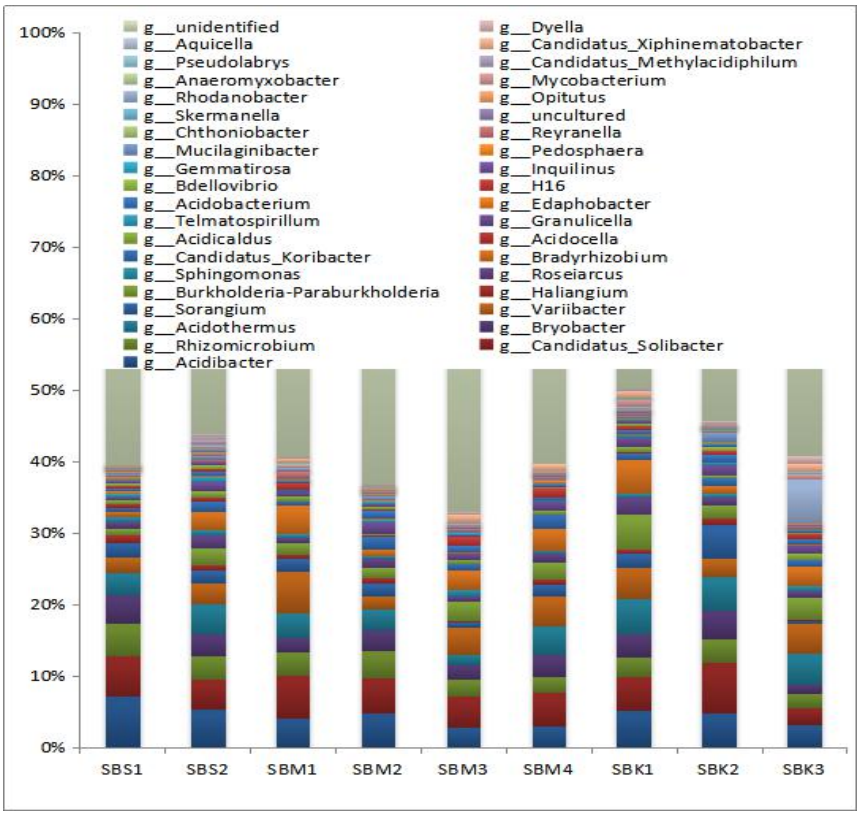

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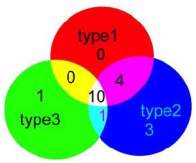

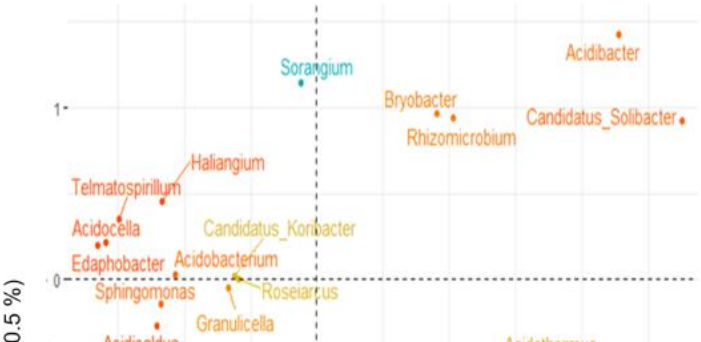

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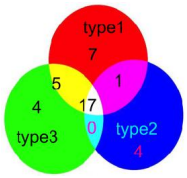

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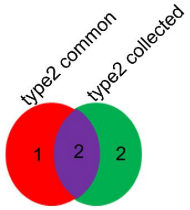

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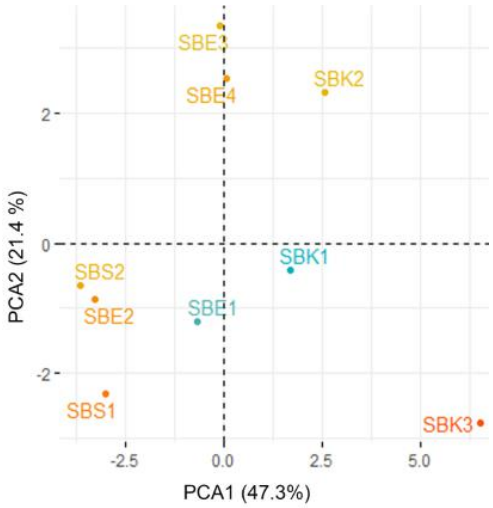

Fig. 3 Diversity analysis of bacterial of three types *Russula* Mycorrhizal rhizosphere in natural *Russula* growth areas

A: The top 20 bacterial genus **c**omposition and abundance in type2 *Russula* symbiosis mycorrhizal rhizosphere and the corresponding genus are in the other two types in natural *Russula* growth areas , Fujian, China

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In the sample name, the first letter s indicates mycorrhizal root soil; first second b stands for bacteria; the third letter different treatment :S indicates sporocarp, E indicates ECM ectomycorrhizal , K indicates control respectively; the fourth number indicates the different biological repetitions of different treatment .

B: The Venny mapping of the common genus of the top 20 bacterial genus in three types *Russula* symbiosis Mycorrhizal rhizosphere

C: The Venny mapping of the collection genus of the top 20 bacterial genus in three types *Russula* symbiosis Mycorrhizal rhizosphere

D: The Venny mapping of the common genus and the collection genus of the top 20 bacterial genus in type2 *Russula* symbiosis Mycorrhizal rhizosphere

E: Analysis of 20 bacterial genus role difference of three types Russula symbiosis mycorrhizal rhizosphere on top 20 bacterial genus of ECM Russula by PCoA

F : **A**nalysis of **c**omposition difference of three types *Russula* symbiosis mycorrhizal rhizosphere on top 20 bacterial genus of ECM *Russula* by PCoA

In E and F:The scales of the horizontal and vertical axes are relative distances and have no practical significance. The contribution rate is the degree of interpretation, and the hypothetical factors can be evaluated and verified.

Table 2 Indicator species of fungi and bacterial community of Russula Mycorrhizal based on the top 20 genus

	Common species in three types	Common species in type1 and type2, Excluding those that are common to all three types	species included exclusively in Type 2,compareing to collecting Type 1,3; and belong to Common in type2
Fungi of ECM	*Tomentella*, *Lactarius*, *Russula*	*Acremonium, Cladophialophora*	*Boletus*

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Russula root			
Fungi of Russula Mycorrhizal rhizosphere	*Russula ,Mortierella, Penicillium Trichoderma*	*Elaphomyces*, *Tomentella*	*Dacryobolus*
Bacterial of Russula Mycorrhizal rhizosphere	*Acidibacter, Acidothermus, Candidatus_Solibacter, Rhizomicrobium, Variibacter, Burkholderia-Paraburkholderia,Roseiarcus, Bradyrhizobium, Candidatus_Koribacter, Granulicella*	*Bryobacter*, *Sorangium, Acidicaldus, Acidobacterium*	*Acidocella, Edaphobacter*

Type2:Russula sporocarp producing; type1 :Russula –infected; type3 :Russula-no-infected; If one species is with one type and without the other type, then the species is assumed as the indicator of.the type based on the top 20 genus.

Interaction of fungi and bacteria with ECM *Russula* root and mycorrhizal rhizosphere

By network net, we analyzed the interaction of the top 20 fungal OTUs of *Russula* rhizosphere soil and ECM *Russula* root, including 7 Russula OTUs(MSFH OTU_1,

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MSFH OTU_3, MSFH OTU_4, MSFH OTU_16, MSFH OTU_21, MSFH OTU_24 and SMFH OTU_1639) (Table S7), five indicator species(MSFH OTU_19 *Elaphomyces*, MSFH OTU_9 *Tomentella*_sp, MSFH OTU_655 *Tomentella*_sp, MSFH OTU_6 *Elaphomyces*, and MSFH OTU_5 *Tomentella*_sp).

Russula, which interacted with other species in positive ways, was the representative and contributed to the main ECM in the community. For example, the interaction result predicted that many fungi had a positive correlation with *Russula* (Fig.4A, Table S4), MSFH OTU_19 (*Elaphomyces*) and MSFH OTU_5 (*Tomentella*_sp) with the *Russula rosea*; MSFH OTU_21, MSFH OTU_655 (*Tomentella*_sp) and MSFH OTU_5 with Russula sp. In all, *Elaphomyces*_sp, *Tomentella*_sp have a positive correlation with *Russula,* combining the previous results that *Elaphomyces* and *Tomentella* were considered indicator species for *Russula* symbiosis in the *Russula* rhizosphere based on the top 20 genera (Tab. 2). Therefore, we further assume that the indicators *Elaphomyces* and *Tomentella* have a positive correlation with *Russula* symbiosis.that

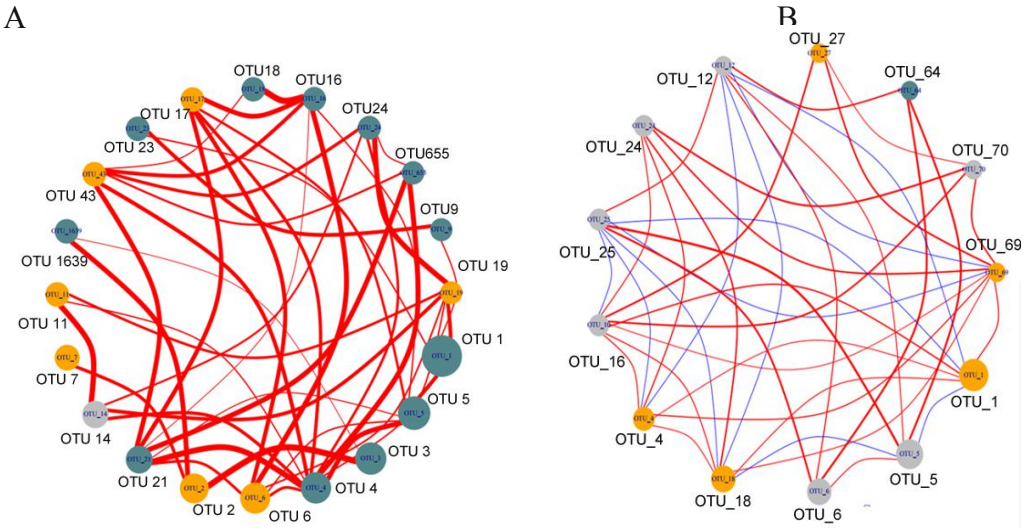

Fig. 4 Interaction of indicator species with the top 20 fungi and bacterial OTUs in

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628 community of *Russula* mycorrhizal

629 A: Interaction of fungi in *Russula* ECM root and *Russula* mycorrhizal rhizosphere

630 B: Interaction of bacterial in Russula mycorrhizal rhizosphere

631 In A and B: The size of the dots represents the abundance, the color of the dots
632 represents the phylum (In A : Orange represents p__Ascomycota, blue represents
633 p__Basidiomycota, gray represents p__Mortierellomycota; in B: gray represents
634 p__Proteobacteria, Orange represents p__Acidobacteria, blue represents
635 p__Actinobacteria), the thickness of the lines represents the magnitude of the
636 correlation, and the red line indicates a positive correlation, the blue color indicates
637 a negative correlation.

638 Meanwhile, we analyzed the relationship of the top 20 bacterial OTUs in *Russula*
639 rhizosphere soil by Network net. Only 15 bacterial OTUs were included in the
640 network net (Fig. 4B, Table S5). Among the top 15 bacterial OTUs, there is 1 OTUs
641 SBH OTU_70 *Sorangium* belonging to indicator species. The network net
642 suggested that SBH OTU_70 was positive with SBH OTU_16 (Acidobacteriaceae),
643 24(Caulobacteraceae), 27(Solibacteraceae), 69(Solibacteraceae). Another indicator
644 species *Acidobacterium* was't within network net . Therefore, we further assumed
645 that *Sorangium* and other OTUs (Acidobacteriaceae, Caulobacteraceae,
646 Solibacteraceae) have a positive correlation with *Russula* .

647 **DISCUSSION**

648 The complete exclusion of other species by any single species could be prevented
649 by either fluctuation in the environment, by seasonal root production or by the
650 presence of microbiome competitive networks, the situation in which no single
651 species is competitively superior to all other species. So, we usually obtain 1 ECM
652 species or 2 ECM species by Sanger sequencing of the root sample with
653 basidiomycete-specific primers. The introduction of high-throughput sequencing
654 techniques, metagenomics or environmental genomics has provided new
655 information on ECM fungal communities by 'barcodes' of ITS regions in several
656 biomes/ecosystems, e.g., truffle grounds (57) and ECM roots in the Svalbard(58).
657 In this study, we found that *Russula* cannot successfully infect roots if the amount

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of *Russula* in the mycorrhizal rhizosphere is less than 10% by MiSeq sequencing (Table 1, part data not shown). Although ECM fungi infection have been detected in nonproductive plots, the amount was not sufficient to shift from vegetative growth to fruit body. *Russula* can form a fruiting body, only when *Russula* infection could be detected by Sanger sequencing of ECM roots, contemporary the relative abundance of its DNA was greater than 60% by MiSeq sequencing. Compared to control, we found significantly more *Russula* DNA in *Russula* sporocarps rhizosphere soil or *Russula* symbiosis rhizosphere soil, above 15% amount of the metagenomics of rhizosphere soil. Zampieri et al.(2012) (49) detected significantly more *T. melanosporum* DNA in truffle productive plots of soil. Zhou et al. (2001) (50) also demonstrated that the development of *S.grevillei* sporocarps is correlated with that of extraradical mycelia, which are distributed in a narrow area. So , we suggest that to the type 2(sporocarps-producing) ,there is congruence of the above-sporocarps and belowground root or mycorrhizospheres soil DNA for *Russula*. However, interestingly, in type 3(uninfected), the amount of *Russula* in the soil is higher than in the root. This may indicate that in the natural growth area of *Russula*, there are a large number of *Russula* propagules in the soil. Whether the *Russula* propagules naturally in the soil of the natural growth area can colonize to the host or not depends on other factors.

Fewer species were detected in *Russula* productive root than in nonproductive root, which is consistent with French *truffle* grounds. *T. melanosporum* grounds also have shown a reduced fungal biodiversity, a reduced presence of both ECM Basidiomycota (57). Truffle-colonization reduced the abundance of some fungal genera surrounding the host tree, such as Acremonium (55) . *Russula* fruiting body decreased the Chao richness bacterial index and Shannon diversity of bacterial index in rhizosphere soil. Simultaneously, *Russula* fruiting body decreased the Chao richness fungi index but no effect on Shannon diversity of fungi index in rhizosphere soil. Warmin (2009)(40) found bacteria adapted to the mycospheres of three or more or just one ECM fungal species was defined as specific selective bacterial. So, we suggest the diversity decreased phenomenon may be related with

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688 the *Russula* sporocarp selection effect, especially to rhizosphere soil bacterial.
689 Temperate forests are generally dominated by Fagaceae, and species in these plant
690 families form mycorrhizae with various phylogenetic clades of ectomycorrhizal
691 fungi(8, 11, 59). Members of the *helotialean* group have recently been identified as
692 the dominant species in the roots of Fagaceae trees in the temperate and subtropical
693 forests of Japan (60, 61). In our study, we found that 3 ECM, *Russula*, *Tomentella*,
694 and *Lactarius*, are the main members in the natural Fagaceae *(Quercus glauca* and
695 *Castanopsis hainanensis*)-dominant *Russula* ground in all the types. This result is
696 consistent with the preference of *Russula* and *Lactarius* for the Fagaceae host. In
697 *Castanopsis*-dominant forest in Japan (62), there are ECM fungi such as *Amanita*
698 (Amanitaceae), *Boletus* (Boletaceae), *Tylopilus* (Boletaceae), *Cortinarius*
699 (Cortinariaceae), *Inocybe* (Inocybaceae), *Lactarius* (Russulaceae), and *Russula*
700 (Russulaceae). The dominant ECM lineages of *Quercus liaotungensis* were
701 *Tomentella*, *Thelephora*, *Cenococcum*, *Russula*, *Lactarius* and *Inocybe*(63). The
702 ectomycorrhizal *Russula*, *Lactarius*, *Cortinarius*, *Tomentella*, *Amanita*, *Boletus* and
703 *Cenococcum* were dominant in the Quercus serrate plot (11). *R. vinosa* grows in
704 tropical and subtropical evergreen forests in southern China dominated by trees of
705 *Castanopsis* spp., and *R. griseocarnosa* grows in forests with Fagaceae (13). Given
706 commercially harvested truffles can establish ectomycorrhizal relationships with
707 different woody host species, and many different combinations of truffle and host
708 species mycorrhizal seedlings are produced in nurseries (64). Our study shows that
709 *Russula* inoculation may establish satisfactory ectomycorrhizal relationships with
710 two indigenous tree species, *Quercus glauca* and *Castanopsis hainanensis.*
711
712 Healthy ECM can support a wide variety of organisms, including a diverse array of
713 fungi other than the dominant ectomycorrhizal symbiont. Some pathogenic fungi,
714 including *Ilyonectria* and *Podospora*, and other competitive mycorrhizal fungi,
715 such as *Hymenochaete*, had significantly lower abundance in the *T.*
716 *borchii*-inoculated root and *Trechispora* and *Humicola*, which were more abundant
717 in the *T. borchii*-inoculated root. There are some notable examples of associations

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718 among *suilloid* fungi and members of the Gomphidiaceae occur within
719 ectomycorrhizal roots (27). Olsson considered *Gomphidius roseus* acted as a
720 parasite of *Suillus bovinus,* the former never occurs without the latter(27) , Based
721 on MiSeq sequencing analysis of the top 20 ectomycorrhizal fungi in *Russula* root,
722 we found *Acremonium*; *Cladophialophora* are associated with ECM
723 *Russula*-Fagaceae roots, and *Boletus* was in association with sporocarp-producing
724 *Russula*. Meanwhile, based on the top 20 genera in *Russula* rhizosphere soil, we
725 found that *Elaphomyces*, and *Tomentella* in association with *Russula* symbiosis;
726 *Dacryobolus* were associated with sporocarp-producing *Russula*. The result will
727 help to find and develop PGPF for *Russula* symbiosis or sporocarp-producing.
728
729 Many studies have addressed the role of soil bacteria in establishing the symbiotic
730 relationship between plants and mycorrhizal fungi (43, 65). We found differences in
731 fungal genus among the three ECM Russula types in rhizosphere soil (Table S 3,
732 Table 2).The interactions of bacteria with the dense hyphal network underneath
733 fungal fruiting bodies have also been addressed (44, 66). Differences in bacterial
734 communities associated with the mycorrhizospheres of *Suillus bovinus*- and
735 *Paxillus involutus*-colonized plants were detected early (67, 68). Frey et
736 al.(1997)(69) reported that specific *Pseudomonas fluorescens* prefer *Laccaria*
737 *laccata*. *P.fluorescens* and *Burkholderia terrae* are exclusively found in the
738 mycosphere soil of *Laccaria proxima* (30, 40). Boersma(2009)(36) found that the
739 mycospheres of basidiomycetous fungi indeed exerted a universal selective effect
740 on the *Pseudomonas* community (i.e., *Pseudomonas poae, P. lini*, *P. umsongensis*, *P.*
741 *corrugata*, *P. antarctica* and *Rahnella aquatilis*); as well as species-specific
742 selective (i.e. *P. viridiflava* and candidatus *Xiphinematobacter americani*). For the
743 selection of the bacteria family *Sphingomonadaceae* by the mycorrhizal fungi *L.*
744 *proxima* and *R. exalbicans*, the mycosphere effect was most prominent in the latter
745 (36). The mycosphere-isolated bacterium *Burkholderia terrae* has been shown to
746 protect its fungal host *Lyophyllum* sp. from several antifungal agents, such as
747 metabolites produced by *P. fluorescens*, as well as from the antifungal agent

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748 cycloheximide (70). Some studies have concentrated on mycorrhization helper
749 bacteria (MHB) in facilitating mycorrhizal colonization of roots in forest nursery
750 environments(43, 71). Rich in bacteria in the mycorrhizal roots, mycorrhizosphere
751 soil and peridium of desert truffles may be used to increase the survival and
752 mycorrhization in the desert truffle plant production system at a semi-industrial
753 scale(72) . The associated bacteria of *Truffle brûlés* are *Pseudomonas* and
754 *Flavobacteriaceae* (73). To achieve successful reforestation, PGPR and MHB were
755 screened to improve the establishment of *Lactarius deliciosus*-Pinus sp.
756 symbiosis(74). In our study, *Acidocella* , *Bryobacter*, *Sorangium, Acidicaldus*,
757 *Edaphobacter* and *Acidobacterium* were indicator species for *Russula* symbiosis in
758 the bacterial community. we also found ECM universal selective
759 *Sphingomonadaceae* work with *Russula* species-specific selective
760 *Acidocella ,Sorangium, Acidicaldus*, *Edaphobacter* and *Acidobacterium* in the soil
761 bacterial community of *Russula* symbiosis (Fig. 3E).Further, by network analysis,
762 *Acidobacteriaceae, Sorangium* and *Acidobacteria* had a positive correlation with
763 *Russula*. There may be further instruction to provide a wide collection of these
764 bacterial associates of *Russula* and to develop *Russula* MHB or Fagaceae PGPR.
765
766 Only a few edible ectomycorrhizal fungal species, such as black truffles or saffron
767 milk caps, can be produced in manufactured orchards. To date, *Russula* fruit bodies
768 are uncultivable. Five *Russula* were used to inoculate *Shorea parvifolia* seeding
769 successfully (75) , and one *Russula* was used to inoculate *Quercus garryana*
770 seeding successfully(76) . Our results will provide instruction to specifically isolate
771 the fungi or beneficial rhizosphere microbes associated with ECM *Russula* from the
772 root or mycorrhizal rhizosphere. Next , we will further need to isolate and culture
773 the microbial communities of the *Russula* root or mycorrhizospheres soil in three
774 types, which will supplement our research findings. The inoculation of these
775 microbes can stimulate establishment and will help to enhance plant growth and
776 promote a change in infected frequency.
777

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Conclusion. The amount of *Russula* DNA is positively correlated with fruiting body and *Russula* mycorrhizae based on the metagenomics of *Russula* root and soil. Fewer fungi species were detected in *Russula*- infected and *Russula* sporocarp root. Fewer bacteria and fungi species were detected in *Russula*- infected and in *Russula* sporocarp rhizosphere soil. *Boletus* is considered as indicator species in Russula-Fagaceae root (*Quercus glauca* and *Castanopsis hainanensis*) for sporocarp-producing *Russula*. The *Russula* sporocarp rhizosphere fungi *Dacryobolu* and *Russula* sporocarp rhizosphere bacteria *Acidocella* is considered as indicator species for sporocarp-producing *Russula*. On the other hand, a number of taxa within *Acremonium* and *Cladophialophora* were identified in Fagaceae root in *Russula* symbiosis. The *Russula* mycorrhizal rhizosphere fungi *Tomentella,* and *Elaphomyces* and the *Russula* mycorrhizal rhizosphere bacteria *Acidocella*, *Bryobacter, Sorangium* and *Acidobacterium* occurred more frequently in association with the ECM genus *Russula*. Understanding the ectomycorrhizal fungal communities inhabiting natural *Russula* growth areas may give us clues about the dynamics of the targeted *Russula* and the possibility of identifying mycorrhizal fungal species that are good indicators of successful *Russula* semicultivation. This reseach may provide novel targeted strategies to improve the establishment of *Russula*-Fagaceae sp. symbiosis and improve *Russula* ascospore productivity and sustainability.

MATERIALS AND METHODS

Sampling . Our study was conducted in areas of *Russula* growth in Jianou, Fujian province, China, in which species of the genus *Russula* are well represented. The dominant trees of the areas are *Quercus glauca* and *Castanopsis hainanensis*. The herb layer is composed of *Podophyllum peltatum*, *Panax stipuleanatus* and *Saxifraga stolonifera*. Fine roots were excavated 2-3 m from the trunk of an adult *Russula* symbiotic tree(Fig. S1A). In addition, another type of fine roots that was clearly connected to *Russula* sporocarp by extraradical mycelia was collected(Fig. S1B,C). We collected three 15-30 cm fine-root segments (containing 100-200 root tips) at a depth of 20 cm (77) . Roots from a single tree were pooled into a single

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808 plastic bag. More than 200 cm^3 of rhizosphere soil was collected around the root
809 samples for analyses. All root and soil samples were stored in a cooler containing
810 several ice bags and transported to our laboratory within 24 h for subsequent
811 analysis.

812 **DNA extraction, amplification, and sequencing.** The collected root samples were
813 washed carefully with tap water. Root tips were preserved in a plastic centrifuge
814 tube at -20°C before DNA extraction. DNA samples were pulverized using liquid
815 nitrogen. Total genomic DNA was extracted from ECM root tips using a modified
816 cetyltrimethylammonium bromide method, which was modified according to Wang
817 et al. (63)(2017). Total genomic DNA of corresponding rhizosphere soil was
818 extracted using a Fast DNA SPIN Kit (MP Bio) for soil according to the kit
819 operation steps.

820 First, the entire range of fungal ITS sequences was amplified from roots using the
821 ECM Basidiomycetes-specific high-coverage primer polymerase chain reaction
822 (PCR) with the primer pair ITS-1F (CTTGGTCATTTAGACGAAGTAA) and
823 ITS-4B (CAGGAGACTTGTACACGGTCCAG) (M. Gardes, 1993), and
824 traditional Sanger sequencing was performed. The sequences were BLAST against
825 the UNITE database/NCBI database (http://www.ncbi.nlm.nih. gov), and taxonomy
826 was assigned to species or genera using sequence similarity criteria of ≥97% for
827 species and 90–97% for genera. Furthermore, DNA of root tips identified as
828 *Russula* and the corresponding *Russula* symbiosis rhizosphere soil were subjected
829 to Illumina MiSeq high-throughput sequencing of ITS sequences to investigate their
830 associated microbiomes, while DNA of root tips with no mycorrhiza was used as a
831 control. The MiSeq sequences were edited, manually corrected in BioEdit 7.0.8 and
832 clustered into species-level operational taxonomic units (OTUs) at 97% sequence
833 similarity for species delimitation using PlutoF (http://unite.ut.ee) in UNITE(80). If
834 no match was found in the PlutoF system, any resulting OTU assignments were
835 individually checked by BLAST against the UNITE database/NCBI database
836 (http://www.ncbi.nlm.nih. gov).

837 To identify the fungi composition , we analyzed 18S ITS1-ITS4 DNA sequences of

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838 ECM rhizosphere root and soil samples using phylogenetic methods, operational
839 taxonomic unit (OTU) delimitations and ordinations to compare species
840 composition in various types of ECM *Russula*. To identify the bacterial
841 composition **,** we analyzed 16S V3-V4 DNA sequences of ECM rhizosphere soil
842 samples using phylogenetic methods, operational taxonomic unit (OTU)
843 delimitations and ordinations to compare species composition in various types of
844 ECM *Russula*.

845 **Statistical analyses.** Chao1 (species richness index) and Shannon (microbial
846 diversity index) indices were analyzed by 97% OTU similarity. ECM fungal
847 richness and the microbial diversity index of each Russula root tip and the
848 corresponding rhizosphere soil of *Russula* symbiosis were calculated using the
849 vegan package and compared by one-way ANOVA. PCoA analyses were based on
850 any distance other than the Euclidean distance using abundance and
851 presence-absence data of the top 20 genera in three types. Differences in
852 community composition among *Russula*-infected samples were visualized by PCoA
853 (79).

854 **Micro-Community diversity analysis and indicator species analysis.** Taxonomic
855 analyses were generated based on the community species abundance (each was
856 above 0.05% of all reads) using Microsoft Excel . The mian fungal genus patterns
857 were determined by taxonomic analysis at different ECM *Russula* mycorrhizal
858 roots. The main fungal and bacterial genus patterns were also observed in the
859 *Russula* mycorrhizal rhizosphere. To assess *Russula* preference, aspects indicator
860 species analysis was carried out by comparing the top 20 genera of three types of
861 root micro-communities. Indicator species of the community of the top 20 genera
862 based on the species presence in the *Russula* sample and absence in the no-*Russula*
863 sample were analyzed by Venny drawing tools
864 (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

865 **Network analyses.** The relationships of *Russula* and other fungi in the *Russula*
866 community were analyzed by Spearman correlation of the absolute top 20 most
867 abundant in all the samples using the igraph, psych software package. Network nets

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868 constructed on the interrelationship result with P>0.05 or |R|<0.4 were filtered. The
869 relationships of bacteria in the *Russula* mycorrhizal rhizosphere were also analyzed
870 by Spearman's correlation.

871 ## SUPPLEMENTAL MATERIAL

872 Supplemental material for this article may be found at

873 **SUPPLEMENTAL FILE 1,** TIFF file, 0.48 MB.

874 **SUPPLEMENTAL FILE 1,** PDF file, 0.22 MB.

875 **SUPPLEMENTAL FILE 1,** PDF file, 0.22 MB.

876 **SUPPLEMENTAL FILE 1,** PDF file, 0.22 MB.

877 **SUPPLEMENTAL FILE 1,** PDF file, 0.17 MB.

878 **SUPPLEMENTAL FILE 1,** PDF file, 0.13 MB.

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Type	Relative Russula OUT abundance (%) in ECM Russula root	Chao1richness of Fungi Community of ECM Russula root1	Shannon of Fungi Community of ECM Russula root	Relative Russula OUT abundance (%) in ECM Russula rhizosphere	Chao1 richness of Fungi Community of ECM Russula rhizosphere	Shannon of Fungi Community of ECM Russula rhizosphere	Chao1 richness of Bacterial Community of ECM Russula rhizosphere	Shannon of Bacterial Community of ECM Russula Rhizosphere
Russula – infected (type1)	28.74 ± 19.31B[1,2]	663.12 ± 171.655b	4.04 ± 1.007b	20.22 ± 4.59B	901.38 ± 32.30a	4.52 ± 0.53a	1010.28 ± 17.29b	7.54 ± 0.10b
Russula sporocarp producing (type2)	66.66 ± 12.92C	170.44 ± 24.68c	2.20 ± 0.19 c	39.23 ± 7.58C	756.87 ± 52.18b	4.56 ± 0.99a	945.81 ± 25.08b	7.49 ± 0.049b
Russula-no-infected (type3)	1.44 ± 1.02A	782.20 ± 102.345a	4.55 ± 0.921a	5.61 ± 1.71A	874.19 ± 41.16a	4.47 ± 0.95a	1099.52 ± 13.14a	7.94 ± 0.13a

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	Common species in three types	**Common species in type1 and type2, Excluding those that are common to all three types**	**species included exclusively in Type 2,compareing to collecting Type 1,3; and belong to Common in type2**
Fungi of ECM Russula root	*Tomentella*, *Lactarius*, *Russula*	*Acremonium, Cladophialophora*	*Boletus*
Fungi of Russula Mycorrhizal rhizosphere	*Russula ,Mortierella, Penicillium Trichoderma*	*Elaphomyces*, *Tomentella*,	*Dacryobolus*
Bacterial of Russula Mycorrhizal rhizosphere	*Acidibacter, Acidothermus, Candidatus_Solibacter, Rhizomicrobium, Variibacter, Burkholderia-Paraburkholderia,Roseiarcus, Bradyrhizobium, Candidatus_Koribacter, Granulicella*	*Bryobacter*, *Sorangium*, *Acidicaldus*, *Acidobacterium*	*Acidocella*

	TYPE2		TYPE1				TYPE3		
sample name	MFS1[A]	MFS2	MFE1	MFE2	MFE3	MFE4	MFK1	MFK2	MFK3
Total number of genus in Intersection	15		6				4		
Total number of genus in Collection	24		46				41		
1	g__Russula	g__Russula	g__Russula	g__unidentified	g__unidentified	g__Russula	g__Tomentella	g__unidentified	g__unidentified
2	g__Tomentella	g__Tomentella	g__Microidium	g__Russula	g__Russula	g__unidentified	g__unidentified	g__Tomentella	g__Trechispora
3	g__Boletus	g__unidentified	g__Cladophialophora	g__Elaphomyces	g__Sebacina	g__Elaphomyces	g__Sporidesmium	g__Lactarius	g__Tomentella
4	g__unidentified	g__Lactarius	g__unidentified	g__Hydnum	g__Hemileccinum	g__Pseudoprobioscispora	g__Lactarius	g__Russula	g__Pseudoprobioscispora
5	g__Lactari	g__Boletus	g__Cytospor	g__Clitocy	g__Elaphomy	g__Tomentella	g__Russula	g__Rhizoderm	g__Xylogone

	us		a	bula	ces			ea		
6	g__Hymenoscyphus	g__Hymenoscyphus	g__Cortinarius	g__Tomentella	g__Trechispora	g__Cladophialophora	g__Talaromyces	g__Phialea	g__Sebacina	
7	g__Clitocybula	g__Cortinarius	g__Archaeorhizomyces	g__Lauriomyces	g__Psathyrella	g__Lactarius	g__Sebacina	g__Trichoderma	g__Lactarius	
8	g__Xylogone	g__Acremonium	g__Phellinus	g__Cladophialophora	g__Lactarius	g__Mortierella	g__Cladophialophora	g__Cladophialophora	g__Talaromyces	
9	g__Acremonium	g__Phellodon	g__Mortierella	g__Myriostoma	g__Tomentella	g__Fusarium	g__Penicillium	g__Thozetella	g__Calvatia	
10	g__Cladophialophora	g__Xylogone	g__Penicillium	g__Phellodon	g__Lasiosphaeria	g__Acremonium	g__Deconica	g__Rhodoveronaea	g__Cephalotheca	
11	g__Cortinarius	g__Cladophialophora	g__Acremonium	g__Xylogone	g__Xylogone	g__Rasamsonia	g__Coprinellus	g__Chaetosphaeria	g__Lysurus	
12	g__Elaphomyces	g__Clitocybula	g__Lactarius	g__Rhodoveronaea	g__Mortierella	g__Sordaria	g__Trichoderma	g__Gliocephalotrichum	g__Hemileccinum	
13	g__Phellodon	g__Thozetella	g__Rhodoveronaea	g__Acremonium	g__Cladophialophora	g__Sporidesmium	g__Inocybe	g__Chalara	g__Pezoloma	
14	g__Thozetella	g__Elaphomyces	g__Fusarium	g__Cortinarius	g__Oidiodendron	g__Hyaloscypha	g__Mortierella	g__Mortierella	g__Cytospora	
15	g__Chaetosphaeria	g__Kazachstania	g__Cryptosporiopsis	g__Oidiodendron	g__Hydnum	g__Nectria	g__Cercophora	g__Oidiodendron	g__Russula	
16	g__Penicillium	g__Issatchenkia	g__Rhizodermea	g__Lactarius	g__Operculomyces	g__Trichoderma	g__Xylogone	g__Chloridium	g__Oidiodendron	
17	g__Phialea	g__Phialea	g__Dactylellina	g__Clavulina	g__Trichoderma	g__Clathrus	g__Clathrus	g__Cortinarius	g__Thozetella	
18	g__Inocybe	g__Chaetos	g__Myrmecri	g__Rhizode	g__Acremoni	g__Antrodiell	g__Dendroc	g__Hymenoscy	g__Genolevuri	

		phaeria	dium	rmea	um	a	lathra	phus	a
19	g__Colleto trichum	g__Thielav ia	g__Xylogone	g__Sebacin a	g__Clitopil us	g__Colletotri chum	g__Fusariu m	g__Sporidesm ium	g__Penicilliu m
20	g__Myrmecr idium	g__Rhodove ronaea	g__Tomentel la	g__Opercul omyces	g__Pezoloma	g__Talaromyce s	g__Elaphom yces	g__Calonectr ia	g__Chaetomium

	TYPE2		TYPE1				TYPE3		
sample name	SFS1	SFS2	SFE1	SFE2	SFE3	SFE4	SFK1	SFK2	SFK3
Total number of genus in Intersection	12		7				7		
Total number of genus in Collection	26		40				36		
1	g__Russula	g__Russula	g__Mortierella	g__Elaphomyces	g__Russula	g__Russula	g__Archaeorhizomyces	g__unidentified	g__unidentified
2	g__Elaphomyces	g__Elaphomyces	g__unidentified	g__Russula	g__unidentified	g__Elaphomyces	g__Penicillium	g__Tomentella	g__Mortierella
3	g__unidentified	g__unidentified	g__Russula	g__unidentified	g__Trichoderma	g__Mortierella	g__unidentified	g__Rhodoveronaea	g__Geminibasidium
4	g__Clitocybula	g__Tomentella	g__Geminibasidium	g__Archaeorhizomyces	g__Tomentella	g__unidentified	g__Russula	g__Lactarius	g__Tomentella
5	g__Tomentel	g__Lactari	g__Tomente	g__Clitocybu	g__Mortier	g__Pseudopro	g__Pseudopro	g__Russula	g__Russula

	la	us	lla	la	ella	boscispora	boscispora		
6	g__Lactarius	g__Clitocybula	g__Elaphomyces	g__Tomentella	g__Elaphomyces	g__Trichoderma	g__Rhodoveronaea	g__Archaeorhizomyces	g__Penicillium
7	g__Archaeorhizomyces	g__Sporidesmium	g__Penicillium	g__Rhodoveronaea	g__Lactarius	g__Tomentella	g__Myrmecridium	g__Cortinarius	g__Saitozyma
8	g__Mortierella	g__Lasiosphaeria	g__Clitocybula	g__Trechispora	g__Penicillium	g__Penicillium	g__Trechispora	g__Corticium	g__Trichoderma
9	g__Penicillium	g__Mortierella	g__Trichoderma	g__Acremonium	g__Saitozyma	g__Sebacina	g__Cladophialophora	g__Mortierella	g__Clavulina
10	g__Cladophialophora	g__Inocybe	g__Saitozyma	g__Mortierella	g__Umbelopsis	g__Lactarius	g__Aspergillus	g__Saitozyma	g__Pseudoproboscispora
11	g__Cortinarius	g__Saitozyma	g__Bifiguratus	g__Microdochium	g__Sporidesmium	g__Chloridium	g__Oidiodendron	g__Microdochium	g__Bifiguratus
12	g__Saitozyma	g__Cladophialophora	g__Lactarius	g__Cookeina	g__Geminibasidium	g__Amplistroma	g__Trichoderma	g__Sympodiella	g__Sebacina
13	g__Coralloidiomyces	g__Penicillium	g__Cladophialophora	g__Cladophialophora	g__Sebacina	g__Fusarium	g__Chloridium	g__Trichoderma	g__Talaromyces
14	g__Rhodoveronaea	g__Trichoderma	g__Chaetomium	g__Trichoderma	g__Lasiosphaeria	g__Microdochium	g__Chaetomium	g__Penicillium	g__Clitopilus
15	g__Dacryobolus	g__Thanatephorus	g__Hypomyces	g__Pseudoproboscispora	g__Inocybe	g__Antrodiella	g__Mortierella	g__Chaetomium	g__Chaetomium
16	g__Trichoderma	g__Microdochium	g__Umbelopsis	g__Penicillium	g__Bionectria	g__Bifiguratus	g__Lactarius	g__Cladophialophora	g__Umbelopsis
17	g__Acremonium	g__Dacryobolus	g__Coralloidiomyces	g__Myrmecridium	g__Cladophialophora	g__Clitopilus	g__Acremonium	g__Clitopilus	g__Sordaria
18	g__Botryoba	g__Acremon	g__Xylogon	g__Saitozyma	g__Chlorid	g__Sordaria	g__Chaetosph	g__Oidioden	g__Oidiodend

	sidium	ium	e		ium		aeria	dron	ron
19	g__Clitopilus	g__Chaetomium	g__Sebacina	g__Hydnum	g__Chaetomium	g__Nectria	g__Fusarium	g__Clavulinopsis	g__Cladophialophora
20	g__Arachnopeziza	g__Talaromyces	g__Chaetosphaeria	g__Chaetomium	g__Staphylotrichum	g__Acremonium	g__Amplistroma	g__Xylogone	g__Xylogone

		TYPE2	TYPE1				TYPE3		
sample name	SBS1	SBS2	SBE1	SBE2	SBE3	SBE4	SBK1	SBK2	SBK3
Total number of genus in Intersection	18		14				12		
Total number of genus in Collection	20		27				26		
1	g__unidentified	g__unidentified	g__unidentified	g__unidentified	g__unidentified	g__unidentified	g__unidentified	g__unidentified	g__unidentified
2	g__Acidibacter	g__Acidibacter	g__Candidatus_Solibacter	g__Acidibacter	g__Candidatus_Solibacter	g__Candidatus_Solibacter	g__Candidatus_Solibacter	g__Candidatus_Solibacter	g__Rhodanobacter
3	g__Candidatus_Solibacter	g__Acidothermus	g__Acidibacter	g__Burkholderia-Paraburkholderia	g__Variibacter	g__Variibacter	g__Variibacter	g__Sorangium	g__Acidothermus
4	g__Rhizomicrobium	g__Candidatus_Solibacter	g__Rhizomicrobium	g__Acidothermus	g__Burkholderia-Paraburkholderia	g__Acidothermus	g__Bradyrhizobium	g__Acidibacter	g__Variibacter

5	g__Bryobacter	g__Rhizomicrobium	g__Bryobacter	g__Candidatus_Solibacter	g__Acidibacter	g__Bradyrhizobium	g__Acidibacter	g__Acidothermus	g__Acidibacter
6	g__Acidothermus	g__Bryobacter	g__Acidothermus	g__Bradyrhizobium	g__Bradyrhizobium	g__Bryobacter	g__Acidothermus	g__Bryobacter	g__Burkholderia-Paraburkholderia
7	g__Variibacter	g__Variibacter	g__Variibacter	g__Variibacter	g__Rhizomicrobium	g__Acidibacter	g__Rhizomicrobium	g__Rhizomicrobium	g__Bradyrhizobium
8	g__Sorangium	g__Bradyrhizobium	g__Sorangium	g__Bryobacter	g__Bryobacter	g__Burkholderia-Paraburkholderia	g__Bryobacter	g__Variibacter	g__Candidatus_Solibacter
9	g__Haliangium	g__Burkholderia-Paraburkholderia	g__Candidatus_Koribacter	g__Rhizomicrobium	g__Acidothermus	g__Rhizomicrobium	g__Sorangium	g__Burkholderia-Paraburkholderia	g__Rhizomicrobium
10	g__Burkholderia-Paraburkholderia	g__Roseiarcus	g__Granulicella	g__Roseiarcus	g__H16	g__Candidatus_Koribacter	g__Burkholderia-Paraburkholderia	g__Granulicella	g__Bryobacter
11	g__Roseiarcus	g__Sorangium	g__Burkholderia-Paraburkholderia	g__Sorangium	g__Candidatus_Xiphinematobacter	g__Sorangium	g__Reyranella	g__Mucilaginibacter	g__Granulicella
12	g__Sp	g__Candidatu	g__Roseiarcus	g__Granulicell	g__Acidobact	g__Granulic	g__H16	g__Candidat	g__Candidat

	hingo monas	s_Koribacter		a	erium	ella		us_Koribact er	us_Xiphinem atobacter
13	g__Br adyrh izobi um	g__Granulic ella	g__Acidobac terium	g__Candidat us_Xiphinem atobacter	g__Sphingom onas	g__Roseiarc us	g__Roseiarc us	g__Bradyrhi zobium	g__Dyella
14	g__Ca ndida tus_K oriba cter	g__Telmatos pirillum	g__Bradyrhi zobium	g__Mycobact erium	g__Candidat us_Koribact er	g__H16	g__Candidat us_Koribact er	g__Roseiarc us	g__Candidat us_Koribact er
15	g__Ac idoce lla	g__Acidical dus	g__Haliangi um	g__Candidat us_Koribact er	g__Granulic ella	g__Candidat us_Xiphinem atobacter	g__Haliangi um	g__Acidobac terium	g__Acidical dus
16	g__Ac idica ldus	g__Haliangi um	g__Mucilagi nibacter	g__Acidical dus	g__Sorangiu m	g__Haliangi um	g__Acidical dus	g__Haliangi um	g__Acidobac terium
17	g__Gr anuli cella	g__Sphingom onas	g__Telmatos pirillum	g__Leptothr ix	g__Roseiarc us	g__Acidical dus	g__Granulic ella	g__Bdellovi brio	g__Sphingom onas
18	g__Te lmato spiri llum	g__Acidobac terium	g__Inquilin us	g__H16	g__Acidical dus	g__Luedeman nella	g__Rhizobiu m	g__Sphingom onas	g__Roseiarc us
19	g__Ed aphob	g__Acidocel la	g__Bdellovi brio	g__Acidobac terium	g__Mycobact erium	g__Acidobac terium	g__Sphingom onas	g__H16	g__Mycobact erium

	acter								
20	g__Acidobacterium	g__Bdellovibrio	g__Acidicaldus	g__Haliangium	g__Dyella	g__Gemmatimonas	g__Candidatus_Xiphinematobacter	g__Dyella	g__H16

Group	MSFOTU_19	MSFOTU_9	MSFOTU_655	MSFOTU_24	MSFOTU_16	MSFOTU_18	MSFOTU_17	MSFOTU_23	MSFOTU_43	MSFOTU_1639	MSFOTU_11	MSFOTU_7	MSFOTU_14	MSFOTU_21	MSFOTU_2	MSFOTU_6	MSFOTU_4	MSFOTU_3	MSFOTU_5	MSFOTU_1
	s__Elaphomyces_sp[1]	s__Tomentella_sp	s__Tomentella_sp	s__Russula_cyanoxantha	s__Russula_sp	s__Trechispora_sp	s__Helotiales_sp	g__Clitocybula	s__Sordariomycetes_sp	s__Russula_sp	o__Chaetosphaeriales;	s__Microidium_phyllanthi	g__Mortierella	s__Russula_sp	f__Archaeorhizomycetaceae	g__Elaphomyces	s__Russula_sp	s__Russula_odorata	s__Tomentella_sp	s__Russula_rosea
SFK1[2]	0.0	0.0	0.0	0.0	0.0	0.0	11.0	0.0	0.0	486.0	0.0	28.0	16.0	0.0	10107.0	0.0	0.0	929.0	0.0	0.0
SFK2	5.0	92.0	144.0	71.0	53.0	2.0	89.0	272.0	30.0	21.0	3.0	0.0	4362.0	94.0	0.0	102.0	29.0	47.0	246.0	659.0
SFK3	0.0	5.0	1387.0	1181.0	25.0	3.0	47.0	3.0	192.0	14.0	0.0	0.0	867.0	48.0	0.0	19.0	14.0	23.0	98.0	4.0

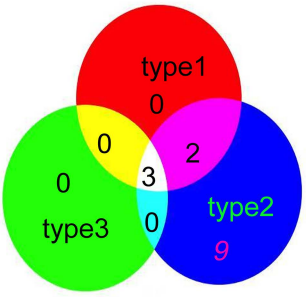
SFE1	1.0	6.0	113.0	5.0	30.0	2.0	96.0	7.0	29.0	43.0	225.0	1.0	32.0	56.0	0.0	28.0	35.0	1734.0	236.0	10.0
SFE2	3.0	11.0	16.0	1.0	22.0	0.0	134.0	895.0	1.0	167.0	0.0	0.0	13.0	50.0	0.0	5968.0	2.0	468.0	833.0	56.0
SFE3	6.0	2.0	33.0	13.0	1637.0	4.0	67.0	40.0	13.0	22.0	0.0	0.0	442.0	2694.0	0.0	622.0	22.0	72.0	801.0	3.0
SFE4	3164.0	3.0	18.0	3326.0	0.0	0.0	31.0	5.0	1.0	20.0	16.0	0.0	1302.0	2.0	0.0	366.0	130.0	36.0	284.0	8.0
SFS1	2.0	754.0	2.0	4.0	0.0	0.0	19.0	2971.0	0.0	7.0	0.0	0.0	22.0	2.0	0.0	79.0	1.0	29.0	10.0	6631.0
SFS2	1.0	6.0	55.0	8.0	3104.0	1.0	100.0	7.0	13.0	12.0	2.0	0.0	50.0	4806.0	0.0	26.0	16.0	32.0	1604.0	3.0

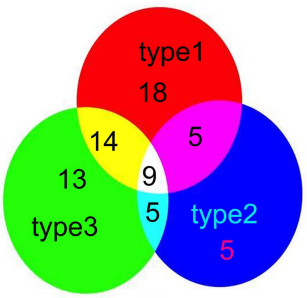
MFK1	1.0	7.0	16.0	8.0	90.0	4.0	1382.0	16.0	14.0	8.0	0.0	0.0	48.0	115.0	0.0	36.0	11.0	21.0	10091.0	4.0
MFK2	13.0	1.0	1782.0	23.0	0.0	0.0	238.0	39.0	2.0	2.0	5116.0	3.0	53.0	2.0	0.0	4.0	61.0	20.0	32.0	6.0
MFK3	0.0	1.0	885.0	136.0	0.0	4740.0	8.0	12.0	3231.0	0.0	0.0	14.0	10.0	0.0	0.0	0.0	2.0	0.0	2.0	4.0
MFE1	0.0	0.0	0.0	0.0	0.0	0.0	98.0	0.0	0.0	3551.0	0.0	6136.0	58.0	0.0	48.0	0.0	0.0	6651.0	1.0	0.0
MFE2	1.0	2.0	10.0	1.0	3.0	8.0	1024.0	741.0	42.0	713.0	0.0	22.0	9.0	3.0	0.0	3740.0	32.0	2773.0	630.0	68.0
MFE3	2.0	0.0	14.0	5.0	32.0	398.0	762.0	36.0	1697.0	104.0	2.0	9.0	89.0	52.0	0.0	482.0	2177.0	366.0	175.0	4.0
M	990.	21.	7.0	58.0	4.0	0.0	105	17.	3.0	109	33.0	1.0	140	2.0	0.0	128	957	196.	236	11.

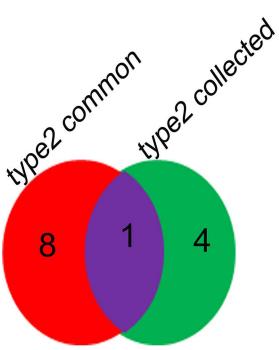
FE4	0	0					2.0	0		.0			.0			.0	2.0	0	.0	0
MFS1	0.0	1453.0	0.0	0.0	0.0	2.0	10.0	116.0	0.0	0.0	0.0	6.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	13431.0
MFS2	0.0	1934.0	0.0	0.0	0.0	0.0	20.0	82.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12658.0

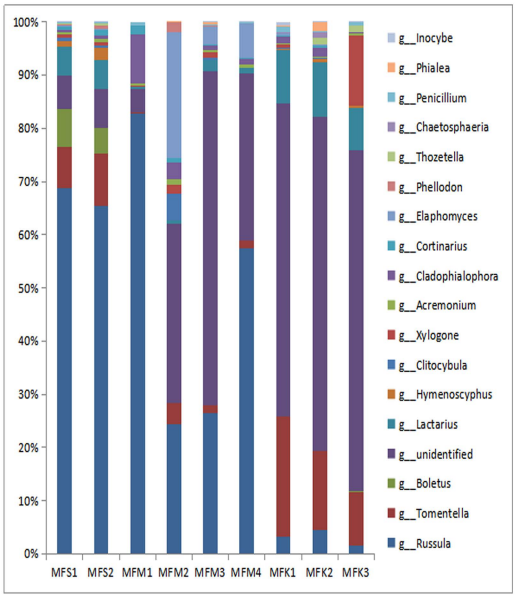
Group	SBOTU_30	SBOTU_69	SBOTU_70	SBOTU_64	SBOTU_27	SBOTU_12	SBOTU_28	SBOTU_35	SBOTU_11	SBOTU_3	SBOTU_41	SBOTU_24	SBOTU_25	SBOTU_10	SBOTU_4	SBOTU_18	SBOTU_6	SBOTU_16	SBOTU_5	SBOTU_1
	g__Rosei arcus	f__Solibacteraceae	g__Sorangium[1]	g__Acidothermus	f__Solibacteraceae_	o__Rhodospirillales	p__Acidobacteria	f__Xanthomonadales_Incertae_Sedis	p__Acidobacteria	p__Acidobacteria	f__Xanthomonadales_Incertae_Sedis	f__Caulobacteraceae	o__Rhodospirillales	f__Rhizobiales_Incertae_Sedis	p__Acidobacteria	f__Acidobacteriaceae_	g__Bradyrhizobium	f__Acidobacteriaceae	g__Variibacter	f__Acidobacteriaceae_
SBK1[2]	516.0	165.0	252.0	315.0	304.0	239.0	157.0	487.0	158.0	231.0	186.0	450.0	547.0	428.0	391.0	496.0	1171.0	769.0	1073.0	567.0
SBK2	175.0	226.0	797.0	181.0	399.0	90.0	275.0	278.0	123.0	642.0	397.0	278.0	273.0	448.0	441.0	618.0	272.0	1358.0	662.0	1375.0
SBK3	182.0	57.0	50.0	380.0	141.0	640.0	51.0	302.0	58.0	333.0	27.0	124.0	647.0	137.0	96.0	283.0	668.0	430.0	1052.0	230.0
S	14	75.	16	277	200	364	187	207.0	617	161	477.0	219	371	212.0	138	172.	996	304.	14	551.

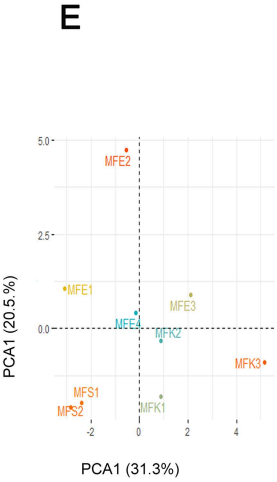
BE1	9.0	0	3.0	.0	.0	.0	.0		.0	.0		.0	.0		.0	0	.0	0	54.0	0
SBE2	163.0	357.0	201.0	144.0	303.0	58.0	180.0	204.0	168.0	377.0	283.0	520.0	52.0	537.0	807.0	1202.0	274.0	794.0	465.0	2770.0
SBE3	148.0	49.0	66.0	119.0	127.0	229.0	729.0	136.0	427.0	363.0	288.0	135.0	572.0	114.0	360.0	313.0	662.0	630.0	971.0	739.0
SBE4	262.0	121.0	161.0	263.0	127.0	341.0	274.0	152.0	674.0	310.0	206.0	198.0	321.0	183.0	439.0	381.0	815.0	917.0	1075.0	907.0
SBS1	129.0	723.0	182.0	163.0	249.0	26.0	72.0	321.0	61.0	110.0	572.0	423.0	35.0	473.0	501.0	1341.0	185.0	468.0	544.0	2126.0
SBS2	264.0	222.0	164.0	204.0	204.0	147.0	217.0	279.0	146.0	98.0	384.0	562.0	170.0	464.0	620.0	842.0	629.0	790.0	740.0	1124.0

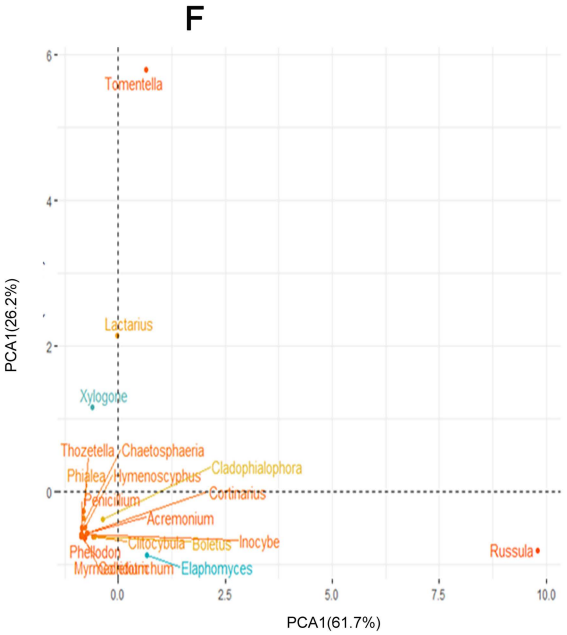

A
type1
0
0
2
0
3
type3
0
type2
9

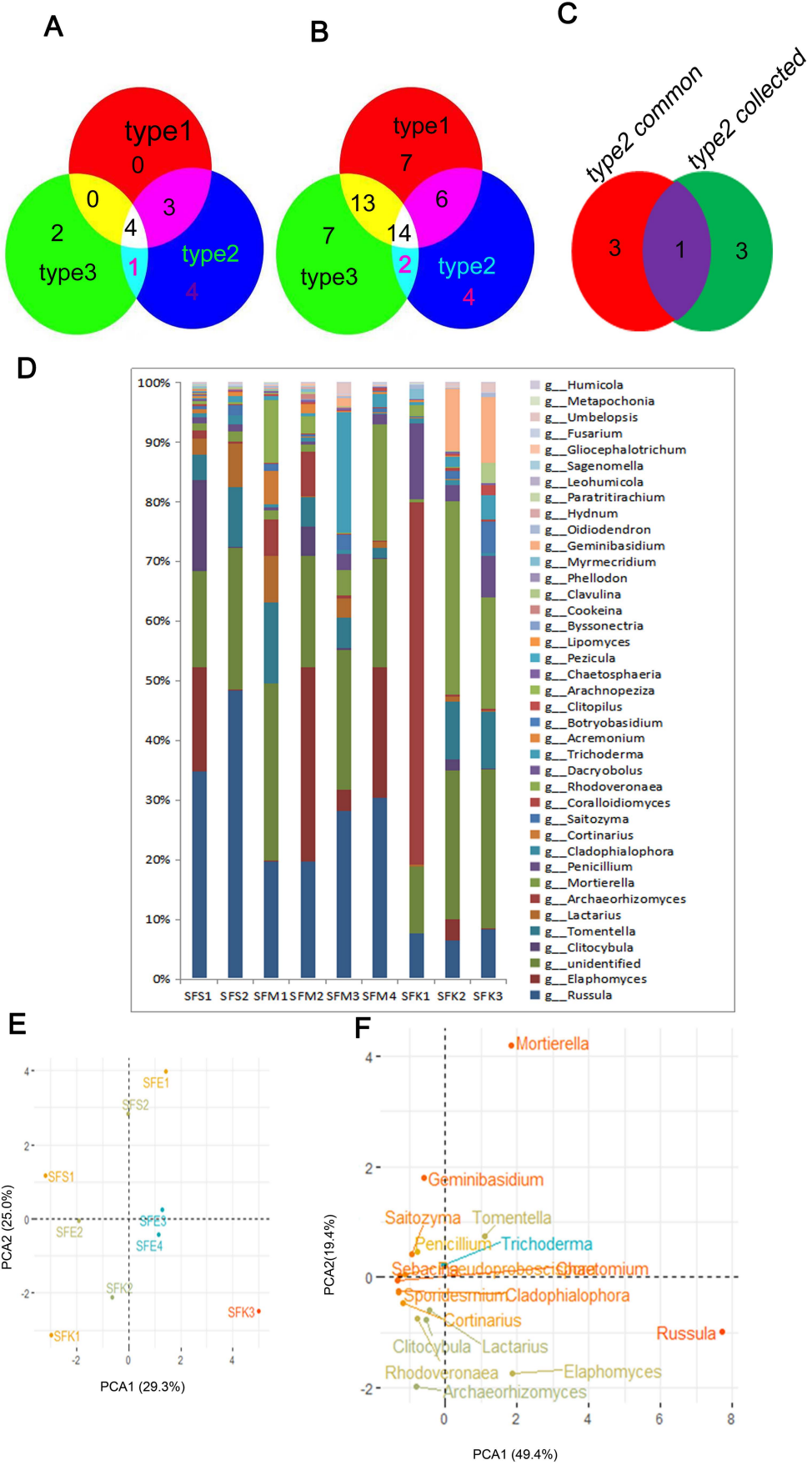

B
type1
18
14
5
13
9
type3
5
type2
5

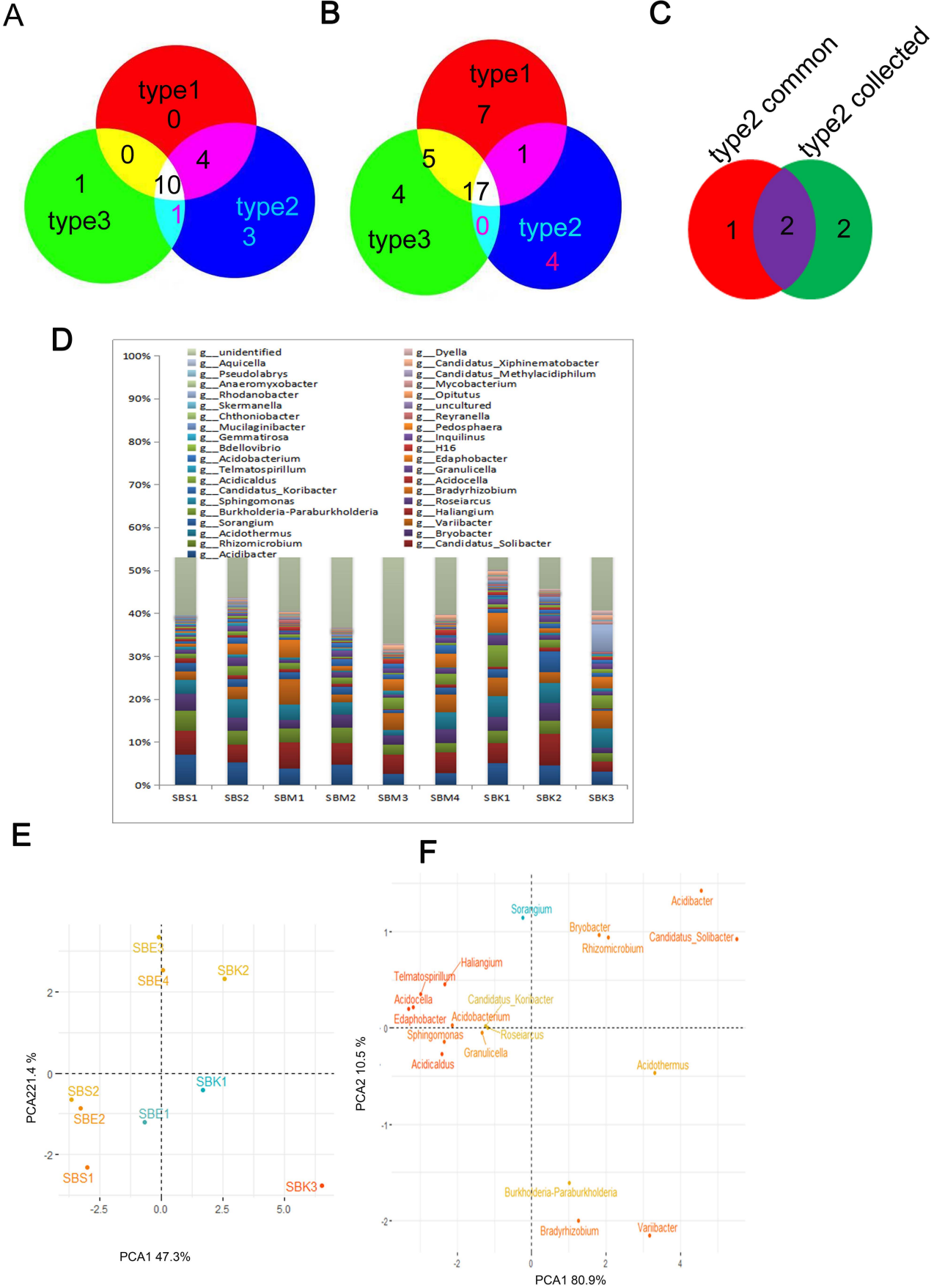

C
type2 common
type2 collected
8
1
4

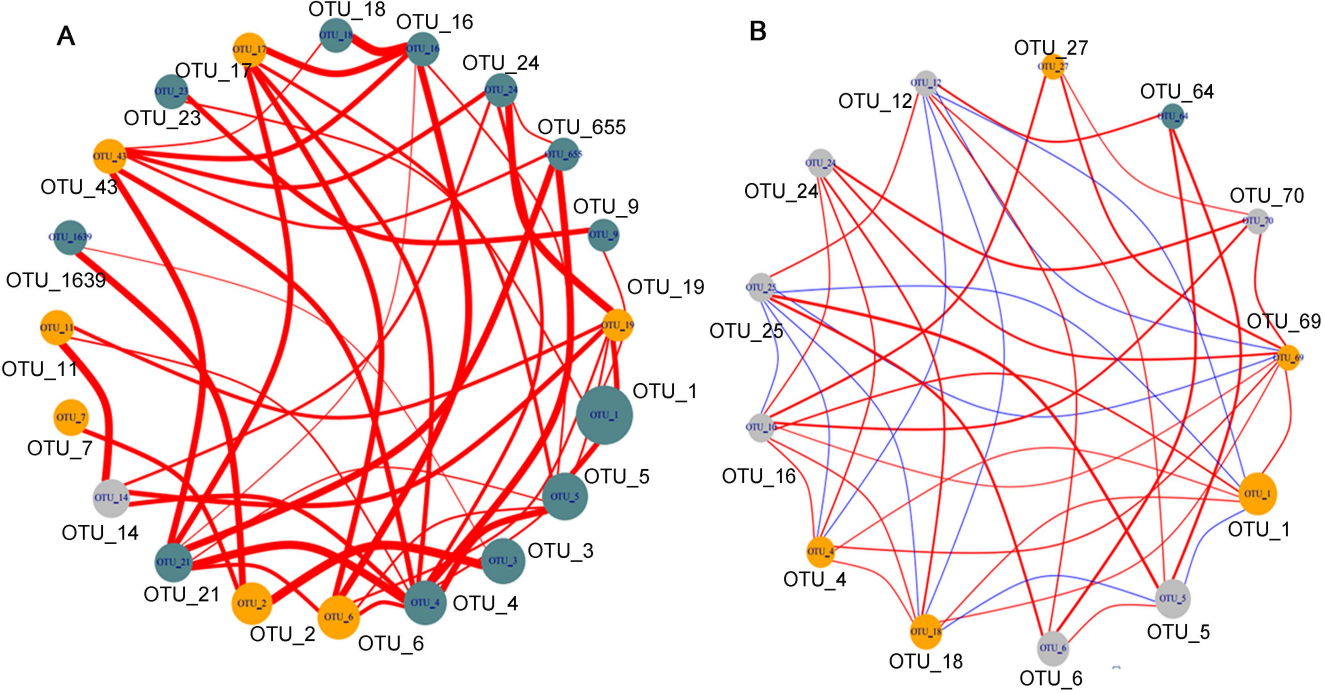

D
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
MFS1
MFS2
MFM1
MFM2
MFM3
MFM4
MFK1
MFK2
MFK3
g__Inocybe
g__Phialea
g__Penicillium
g__Chaetosphaeria
g__Thozetella
g__Phellodon
g__Elaphomyces
g__Cortinarius
g__Cladophialophora
g__Acremonium
g__Xylogone
g__Clitocybula
g__Hymenoscyphus
g__Lactarius
g__unidentified
g__Boletus
g__Tomentella
g__Russula


E
5.0
2.5
0.0
MFE2
MFE1
MFE3
MFE4
MFK2
MFK3
MFS1
MFS2
MFK1
-2
0
2
4
PCA1 (20.5%)
PCA1 (31.3%)


F
6
4
2
0
Tomentella
Lactarius
Xylogone
Thozetella
Chaetosphaeria
Phialea
Hymenoscyphus
Cladophialophora
Penicillium
Cortinarius
Acremonium
Clitocybula
Boletus
Inocybe
Phellodon
Elaphomyces
Russula
0.0
2.5
5.0
7.5
10.0
PCA1(26.2%)
PCA1(61.7%)


A
type1
0
0
3
2
4
type3
1
type2
4
B
type1
7
13
6
7
14
type3
2
type2
4
C
type2 common
type2 collected
3
1
3
D
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
SFS1 SFS2 SFM1 SFM2 SFM3 SFM4 SFK1 SFK2 SFK3
g__Humicola
g__Metapochonia
g__Umbelopsis
g__Fusarium
g__Gliocephalotrichum
g__Sagenomella
g__Leohumicola
g__Paratritirachium
g__Hydnum
g__Oidiodendron
g__Geminibasidium
g__Myrmecridium
g__Phellodon
g__Clavulina
g__Cookeina
g__Byssonectria
g__Lipomyces
g__Pezicula
g__Chaetosphaeria
g__Arachnopeziza
g__Clitopilus
g__Botryobasidium
g__Acremonium
g__Trichoderma
g__Dacryobolus
g__Rhodoveronaea
g__Coralloidiomyces
g__Saitozyma
g__Cortinarius
g__Cladophialophora
g__Penicillium
g__Mortierella
g__Archaeorhizomyces
g__Lactarius
g__Tomentella
g__Clitocybula
g__unidentified
g__Elaphomyces
g__Russula
E
SFE1
SFS2
SFS1
SFE2
SFE3
SFE4
SFK2
SFK3
SFK1
PCA2 (25.0%)
PCA1 (29.3%)
F
Mortierella
Geminibasidium
Saitozyma
Tomentella
Penicillium
Trichoderma
Cladophialophora
Cortinarius
Russula
Clitocybula
Lactarius
Rhodoveronaea
Elaphomyces
Archaeorhizomyces
PCA2(19.4%)
PCA1 (49.4%)


A
type1
0
0
4
1
10
type3
1
type2
3
B
type1
7
5
1
4
17
type3
0
type2
4
C
type2 common
type2 collected
1
2
2
D
g__unidentified
g__Aquicella
g__Pseudolabrys
g__Anaeromyxobacter
g__Rhodanobacter
g__Skermanella
g__Chthoniobacter
g__Mucilaginibacter
g__Gemmatirosa
g__Bdellovibrio
g__Acidobacterium
g__Telmatospirillum
g__Acidicaldus
g__Candidatus_Koribacter
g__Sphingomonas
g__Burkholderia-Paraburkholderia
g__Sorangium
g__Acidothermus
g__Rhizomicrobium
g__Acidibacter
g__Dyella
g__Candidatus_Xiphinematobacter
g__Candidatus_Methylacidiphilum
g__Mycobacterium
g__Opitutus
g__uncultured
g__Reyranella
g__Pedosphaera
g__Inquilinus
g__H16
g__Edaphobacter
g__Granulicella
g__Acidocella
g__Bradyrhizobium
g__Roseiarcus
g__Haliangium
g__Variibacter
g__Bryobacter
g__Candidatus_Solibacter
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
SBS1
SBS2
SBM1
SBM2
SBM3
SBM4
SBK1
SBK2
SBK3
E
SBE3
SBE4
SBK2
SBK1
SBS2
SBE2
SBE1
SBS1
SBK3
PCA221.4 %
PCA1 47.3%
F
Sorangium
Acidibacter
Bryobacter
Candidatus_Solibacter
Rhizomicrobium
Haliangium
Telmatospirillum
Acidocella
Candidatus_Koribacter
Edaphobacter
Acidobacterium
Sphingomonas
Roseiarcus
Granulicella
Acidicaldus
Acidothermus
Burkholderia-Paraburkholderia
Bradyrhizobium
Variibacter
PCA2 10.5 %
PCA1 80.9%


A
OTU_18
OTU_16
OTU_17
OTU_24
OTU_23
OTU_655
OTU_43
OTU_9
OTU_1639
OTU_19
OTU_11
OTU_1
OTU_7
OTU_5
OTU_14
OTU_3
OTU_21
OTU_4
OTU_2
OTU_6
B
OTU_27
OTU_12
OTU_64
OTU_24
OTU_70
OTU_25
OTU_69
OTU_16
OTU_1
OTU_4
OTU_5
OTU_18
OTU_6

A Ectomycorrhizal *Russula*

B Extramatrical mycelium of *Russula*

C *Russula* and extramatrical mycelium of *Russula*