Arbuscular Mycorrhizal Fungi Co-Colonizing on A Single Plant Root System Recruit Distinct Microbiomes

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Research

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Arbuscular mycorrhizal fungi co-colonizing on a single plant root system recruit distinct microbiomes

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Abstract

Background: Plant roots are usually colonized by various arbuscular mycorrhizal (AM) fungal species which vary in morphological, physiological and genetic traits and constitute the mycorrhizal nutrient uptake pathway (MP) in addition to roots. Simultaneously, the extraradical hyphae of each AM fungus is associated with a community of bacteria. However, whether the community structure and function of microbiome on the extraradical hyphae would differ between the AM fungal species are mostly unknown.

Methods: In order to understand the community structure and the predicted functions of the microbiome associated with different AM fungal species, a split-root compartmented rhizobox culturing system, which allowed us to inoculate two AM fungal species separately in two root compartments was used. We inoculated two separate AM fungal species combinations, *Funneliformis mosseae* (*F.m*) and *Gigaspora margarita* (*G.m*), *Rhizophagus intraradices* (*R.i*) and *G. margarita,* on a single root system of cotton. The hyphal exudate fed active microbiome was measured by combining ^{13}C-DNA stable isotope probing with Miseq sequencing.

Results: We found different AM fungal species, that were simultaneously colonizing on a single root system, hosted distinct active microbiomes from one another. Moreover, the predicted potential functions of the different microbiomes were distinct.

Conclusion: We conclude that the arbuscular mycorrhizal fungi component of the system is responsible for the recruitment distinct microbiomes in the hyphosphere. The potential significance of the predicted functions of the microbiome ecosystem services is discussed.

Key words: Arbuscular mycorrhizae; ^{13}C-DNA-SIP; Hyphosphere; Microbiome; COG; Hyphal exudates; Mycorrhizal pathway.

Background

The plant- arbuscular mycorrhizal (AM) fungi symbiosis has existed for over 460 Mya [1]. Consequently, over 80% of terrestrial plants form a symbiosis with arbuscular mycorrhizal (AM) fungi for efficient nutrient uptake, or to confer resistance to stress [2]. Exploitation of these symbioses is of high environmental and economic value [3]. Like plant roots, AM fungi produce large networks of extraradical hyphae in the soil, release carbon and recruit free-living soil microbes to colonize the hyphae [4-7]. In recent years, an intimate cooperative relationship between AM fungal hyphae and bacteria has been observed, supported by multiple lines of evidence such as microscopic observations [8] and molecular analyses [5]. Bacteria associated with AM fungi (hyphosphere) have been identified as the third component of the plant-AM fungi symbiosis because of the critical role they play in mycorrhizal function [3,6,9,10]. Revealing the secrets of hyphosphere microbiomes is essential for better understanding of the belowground ecosystem.

Many factors such as soil pH and its spatial structure have been identified to influence the bacterial community associated with plant roots, while AM fungi was identified as the major factor which determined it [11]. In natural and agricultural systems, the root system of a mycorrhizal plant is usually simultaneously colonized by diverse AM fungal species [12]. The co-colonizing AM fungi have different morphological, physiological and genetic characteristics [13-18]. The coexisting AM fungal species show different contributions to the growth and P uptake of the host plant [15]. For example, *Glomus intraradices* can rapidly colonize available P patches beyond the root surface and transport significant amounts of P towards the roots, while *G. margarita* has been shown to provide P benefits to the plants by forming dense mycelium networks close to the roots where remaining soil P was less available [15].

In addition, recent decoding of the whole-genome sequence of AM fungi suggest that there is large variation in the genetic control of functions [16], e.g. *G. rosea* contains a much greater secretome size and more secreted proteins (SSP) than *Rhizophagus* sp. [16]. Collectively, the above morphological, physiological and genetic differences indicate that the hyphal exudates of AM fungal species are likely to be different, which in turn are likely to lead to differences in the hyphosphere microbiome community structure and function. However, at present no direct evidence exists which show the difference between fungal species co-colonizing on a single plant root system. Therefore, to uncover such difference is fundamental for understanding the central question in fungi-bacteria interaction research: how bacteria and mycorrhizal fungi associate and become mutually beneficial neighbors [3].

Several factors may affect the results of hyphosphere microbial community composition in plant-AM fungi-soil system. First, plant root exudates are an important factor in the recruitment of soil microbial community. In order to get direct evidence of the effect of hyphae exudates on hyphosphere microbiome characteristics, it is essential to separate their influence away from that of the root exudates. Second, the vitality of AM fungal hyphae is important. Previous studies have shown that soil bacteria differ in their ability to colonize vital and nonvital hyphae and that this can also be influenced by the arbuscular mycorrhizal fungal species involved [19]. Therefore, a method that can test the vital and nonvital hyphae is necessary to identify the hyphosphere microbiome. Third, the feedback effects of plant on the growth of AM fungus due to changes in plant physiology induced by the fungus [20,21] is critical. In the past, split-root methods were used to quantify C allocation to different AM fungal species co-colonizing on a single root system of plant [22] in order to assess this factor.

In this study, we hypothesized that the different AM fungal species that colonized

on a single root system would recruit distinct microbiomes. To test our hypothesis, we developed a new integrated approach to avoid the above influences. We grew cotton (*Gossypium hirsutum* L.) plants in a split-root and compartmented rhizobox in which a buffer zone was set to prevent root exudate diffusing into the hyphal compartment and to avoid feedback effects. We inoculated two different AM fungal species combinations *Funneliformis mossea*/*Gigaspora margarita* (*F.m*/*G.m*) or *Rhizophagus intraradices*/*Gigaspora margarita* (*R.i*/*G.m*) to both root compartments. We used $^{13}CO_2$ to pulse label the plant-AM fungi-hyphae associated bacteria during the last week before harvest and tested active hyphal associated microbiomes by ^{13}C-DNA-SIP （stable isotopic probing） method and Miseq high-throughput sequencing.

Results

Mycorrhizal colonization

We used the DNA copy number in roots to indicate the colonization of each AM fungal species. Based on the principles of qPCR, any measurement that is less than 100 copies can be considered background and indicative of a lack of presence of mycorrhizal DNA [18]. In NM controls in both Exp 1 and Exp 2, the root AM fungal DNA copy number was below this threshold, indicating that no AM fungus was detected in the roots. Both species of AM fungi were able to colonize the root system of the same plant effectively at the same time. In Exp 1, after inoculation with *F. mosseae*, the root AM fungal DNA copy number significantly increased to 10^7; and inoculation with *G. margarita* increased the AM fungal DNA copy number to 10^5, which was significantly less than that of the concomitant inoculation with *F. mosseae* (P<0.01) (Fig. 2a). In Exp 2, after inoculation with *R. intraradices*, the root AM fungal DNA copy number significantly increased to 10^7; and inoculation with *G. margarita* increased the AM fungal DNA copy number to 10^5, significantly less than that of

concomitant inoculation with *R. intraradices* (P<0.01) (Fig. 2a).

Hyphal length density in HCs soil

In the NM control in both Exp 1 and Exp 2, less than 0.6 m g^{-1} soil of hyphae was detected, implying there were some saprotrophic fungi in compartments. In Exp 1, the hyphal length density of *F. mosseae* was more than 6 m g^{-1} soil, while the density of *G. margarita* was about 3 m g^{-1} soil. *G. margarita* produced significantly (P<0.001) less hyphal length than *F. mosseae* (Fig. 2b). Similarly, in Exp 2, the hyphal length density of *R. intraradices* was 6 m g^{-1} soil while that of *G. margarita* was only 3 m g^{-1} soil, significantly (P<0.001) less than that of *R. intraradices* (Fig. 2b).

Biomass, P concentration and P content of shoot

The cotton plants grew well after being transplanted into the split-root microcosm (See Fig. S2). At harvest, the shoot biomass and P concentration and P content data of all inoculation treatments were significantly (P<0.01) greater than those of their NM control treatments, respectively (Table 1).

^{13}C incorporation of HCs soil and bacteria

The DNA of targeted bacterial populations in the hyphosphere was successfully labeled by ^{13}C. In the NM control, the isotopic signature (δ^{13}C) of hyphosphere soil was consistent with the atmospheric concentration (approximate -20‰). The isotopic signatures in the HCs of inoculated treatments were greater than that of NM control (Fig. 2c). In addition, inoculation with *F. mosseae* and *R. intraradices* resulted in much greater ^{13}C abundance than that of *G. margarita* in Exp 1 and Exp 2, respectively (Fig. 2c). The incorporation of ^{13}C into bacterial DNA in the hyphosphere soil was corroborated by parallel incubation of microcosms labeled with ^{12}C. The gradients in all ^{12}C labeled soil after seven days clearly showed peaks of bacterial DNA in a 'light' DNA fraction. In contrast, the bacterial DNA in all ^{13}C labeled soil had apparently

147 shifted toward ‘heavier’ buoyant densities (Fig. S3).

148 **Taxonomic profiling of bacteria associated with AM fungal hyphae**

149 The DNA from the selected fractions shown in Figure S2 was sequenced using a
150 high-throughput MiSeq PE 300 platform. After quality filtering and standardizing of
151 the raw data, a dataset of 1989255 high-quality sequences with an average length of
152 439 bp and over 24433 reads per sample was generated. At 97% similarity, the number
153 of operational taxonomic units (OTUs) ranged from 505 to 836, depending on the
154 sample (Fig S4). The microbiome of ^{13}C-labeled samples was considered as the active
155 one, which were influenced by the hyphae directly [23]. So, the following analyses were
156 all based on the ^{13}C-labeled active samples.

157 **The effect of AM fungi hyphae on soil microbiome**

158 After aligning the OTUs with the Greengenes database, the soil microbial
159 community was classified into phylotypes consisting of 10 dominant phyla and others.
160 The dominant taxa included Proteobacteria, Actinobacteria, Firmicutes and
161 Gemmatimonadetes, Bacteroidetes, Chloroflexi, Acidobacteria, Cyanobacteria,
162 Planctomycetes and Fusobacteria, which contributed to over 95% of the whole
163 community in all conditions (Fig. S5). There was a significant difference in the
164 abundance of some taxa compared with NM control after inoculation. However, the
165 difference in taxa abundance was dependent on the AM fungal species (Fig. S5). For
166 example, compared to the NM control, (i) the hyphosphere of *F. mosseae* contained a
167 greater abundance of Actinobacteria and Gemmatimonadetes, but contained fewer
168 Proteobacteria, Bacteroidetes, Acidobacteria and Planctomycetes. (ii) the hyphosphere
169 of *R. intraradices* contained a greater abundance of Actinobacteria and Firmicutes, but
170 contained fewer Proteobacteria and Bacteroidetes. (iii) the hyphosphere of *G. margarita*
171 contained a greater abundance of Proteobacteria, Cyanobacteria and Fusobacteria, but

fewer Gemmatimonadetes, Chloroflexi, Acidobacteria and Planctomycetes (Fig. S5). In addition, the PCA analysis also showed the community structure of the inoculated hyphal compartments was different to the NM control (Fig. S6).

The difference between microbiome diversity associated with the hyphae of different AM fungi

In Exp 1, there was no difference was observed in OTUs number of *F. mosseae* hyphosphere microbiome than that of *G. margarita*, while in Exp 2, 100 more OTUs were observed in *R. intraradices* hyphosphere microbiome than that of *G. margarita* (Fig. S4). In addition, there was a significant difference in the abundance of different taxa between different AM fungal species (Fig. 3 and 4). At the phylum level, the abundance of Proteobacteria, Cyanobacteria and Fusobacteria in the hyphosphere of *G. margarita* was much greater than that of *F. mosseae* and *R. intraradices*, both in Exp 1 and Exp 2. However, *G. margarita* exhibited a smaller abundance of Actinobacteria, Gemmatimonadetes and Planctomycetes (Fig. 3). There was no significant difference in the abundance of Firmicutes, Chloroflexi, Bacteroidetes, and Acidobacteria, between *F. mosseae* and *G. margarita* or between *R. intraradices* and *G. margarita* in Exp 1 and Exp 2, respectively (Fig. 3).

At the genus level, there were a total of 733 genera observed in this study. We only considered the genus whose abundance was over 1% as the dominant taxa. In Exp1, 16 genera were identified as dominant taxa both in *F. mosseae* and *G. margarita* hyphosphere microbiome. However, only 7 of them were both dominant in two different AM fungal hyphosphere. In Exp2, 18 genera and 15 genera were identified as dominant in *R. intraradices* and *G. margarita* hyphosphere, respectively. Only 5 genera were both dominant in these two different AM fungal hyphosphere in Exp 2. Of all the genera, 92 genera which contained most of the dominant genera were observed as being

different between the microbiomes associated with *F. mosseae* and *G. margarita* extraradical hyphae in Exp 1, this represented approximately 70% of total abundance. Likewise, there were 108 genera which contained most of the dominant genera were observed as being different between *G. margarita* and *R. intraradices* in Exp 2, representing over 80% of total abundance in the *R. intraradices* hyphosphere and over 50% in the *G. margarita* hyphosphere, respectively (Fig. 4). In accordance with this, the PCA analysis results demonstrated that there was a significant difference between *F. mosseae* and *G. margarita* or *R. intraradices* and *G. margarita* community structure in Exp 1 and Exp 2, respectively (Fig. 6).

The Cluster of Ortholog Genes (COG) functional pathway prediction

Twenty-two COG pathways were predicted through 16S rDNA sequencing of ^{13}C labeled samples. These included all bacterial growth processes such as reproduction, organic or inorganic nutrient metabolism, signaling and immunity (Fig. 7). Eleven COG functional pathways, which contained over half of all the pathways obtained significantly different abundance between *F. mosseae* and *G. margarita* in Exp1 (Fig. 7). In detail, the relative abundance of Amino acid transport and metabolism, Cell motility, Coenzyme transport and metabolism, General function prediction only, Intracellular trafficking, secretion, and vesicular transport and Transcription were greater in the *G. margarita* hyphosphere microbiome. While the relative abundance of Carbohydrate transport and metabolism, Defense mechanisms, Energy production and conversion, Secondary metabolites biosynthesis, transport and catabolism and Translation, ribosomal structure and biogenesis were much more prevalent in *F. mosseae* hyphosphere microbiome. Fifteen COG pathways showed a significant difference between *R. intraradices* and *G. margarita*, while eight of them were greater in the *R. intraradices* hyphosphere (Fig. 7). In detail, Cell cycle control, cell division,

chromosome partitioning, Cell motility, Coenzyme transport and metabolism, Inorganic ion transport and metabolism, Intracellular trafficking, secretion, and vesicular transport, Posttranslational modification, protein turnover, chaperones, Replication, recombination and repair and Signal transduction mechanisms were much more prevalent in *G. margarita* hyphosphere microbiome. While the relative abundance of Carbohydrate transport and metabolism, Cytoskeleton, Defense mechanisms, Lipid transport and metabolism, RNA processing and modification, Secondary metabolites biosynthesis, transport and catabolism and Transcription were much greater in *R. intraradices* hyphosphere microbiome. Most interestingly, carbohydrate transport and metabolism pathways represented over 6% of all the results, and *G. margarita* exhibited a smaller abundance of these pathways than *F. mosseae* and *R. intraradices* in Exp 1 and Exp 2, respectively (Fig. 7).

Discussion

Validation of a novel method for separating out the impact of AM fungi on the soil microbiome

Traditionally, mycorrhizal colonization is measured by staining and microscopic observation methods [24]. In contrast, in this study we used q-PCR to quantify the DNA copy number to indicate mycorrhizal fungi colonizing statue with species specific 18S rRNA primers. There is a background threshold of 100 copies in the AM fungi DNA q-PCR process that dictates the presence or absence of AM fungi [18]. Our results suggested that all inoculated treatments have many orders of magnitude more DNA copies than those of control treatments (Fig. 2a). In addition, no other non-targeted AM fungus was found in any sample through PCR using AM fungal specie specific primers. Such results suggest that all inoculated AM fungi were well colonized in the split-root system of cotton without contamination.

In this study, we compared the bacterial community that associated with the hyphae (representing the hyphosphere microbiome) with the bacterial community in the soil collected from HCs of non-mycorrhizal treatments (representing the bulk soil). As the diameter of AM fungal hyphae is so small that is difficult to separate soil particles from the hyphae, we therefore used the bacteria that were tightly colonizing on the hyphal surface to indicate the status of the hyphosphere bacterial community.

To avoid any influence of root exudates on the measurements, we set a 1 cm wide buffer zone in which we added sterilized mixture of glass beads and fine clay soil which was sieved through 30 μm nylon mesh. Our results showed that $\delta^{13}C$ of the HCs soils of the control treatments were the same as the background, suggesting no direct influence from root exudate on the microbiome community in HCs. Therefore, all differences between hyphosphere and bulk soil or between the different AM fungal species can be attributed to the effects of hyphal exudation.

As the turnover rate of AM extraradical hyphae is fast [25], both vital and nonvital hyphae exist simultaneously, importantly it is thought that the bacterial communities associated with these two types of hyphae may differ [19]. To avoid these influences, we took a seven-day-pulse labelling approach in the last week before harvesting, which ensures that, all the ^{13}C labelled extraradical mycelium were vital, because the potential turnover time of AM fungal hyphae is 5-6 days [25]. We assume nonvital hyphae will not consume the ^{13}C labelled carbohydrates because the senescent hyphae form sepat to cease the protoplasm flow in hyphae. Therefore, the atom percent of ^{13}C of the samples in HCs indicated the allocation of photosynthetic products to vital extraradical hyphae and hyphae associated with soil particle and bacteria, and the ^{13}C-DNA-SIP identified hyphosphere microbiome were active hyphae exudates consumers.

The influence of AM extraradical hyphae exudates on biophysical distribution

of soil microbial community and biodiversity

Arbuscular mycorrhizal fungi produce a large network of extraradical hyphae in soil and provide a carbon rich habitat for soil microbes [5,6], which induces colonization of diverse groups of bacteria forming the hyphosphere [7,26,27]. Our current study not only further supports those previous findings but provided novel findings as well. First, the differences in q-PCR (Fig. 2a) and plant biomass (Table 1) results between *F.mosseae*/*G.margarita* and NM in Exp 1 or *R.intraradices*/*G.margarita* and NM in Exp 2 indicated that all AM fungal species colonized roots of cotton and played a role in promoting plant growth. Second, we successfully separated the active bacteria that consumed hyphal exudates by ^{13}C-DNA-SIP plus Miseq sequencing methods (Fig. S3). Compared to bulk soil, we found that only part of the soil microbiome was ^{13}C-labelled on the hyphae of the AM fungi which we defined as the active hyphosphere microbiome (Fig. S4). Third, the co-colonizing AM fungi all formed a unique bacterial community around the extraradical mycelium (Fig S3 and Fig. S5). Our observations help us understand biophysical mechanisms which dictate the heterogeneous distribution of the microbiome at the micro-scale [28-30]. Our current finding provides new and direct evidence that shows that AM fungal hyphae, most likely through their exudates, are one of the major driving forces for formation of the bacteria mosaic at micrometer scale in soil. As AM fungi use up to 20% of plant photosynthesis products and form several meters to tens of meters of hyphae in one gram of soil [31], understanding of such mechanisms have significance even within the context of the global soil microbial biodiversity pattern.

Co-colonizing AM fungal species recruited different active hyphosphere microbiome community

Although previous studies have shown that a range of AM fungal species, which

298 are different in morphological structure, hyphal distribution pattern and their metabolic
299 traits, can simultaneously colonize a single root system [15,32,33]. Whether or not these
300 fungi recruit different microbiome with their own preference is still an open question.
301 We hypothesized that any difference in microbiome community structures
302 between the two HCs in Exp 1 and Exp 2 can be attributed to the differences in traits of
303 excretion of exudates between the two AM fungal species. Our ^{13}C-DNA-SIP plus
304 pyrosequencing results supported the hypothesis. First, different AM fungal species
305 produced differing amounts of hyphae in HCs (Fig. 2b). Compared to *G. margarita*, the
306 HCs of both *F. mosseae* and *R. intraradices* contained a greater ^{13}C abundance. Second,
307 there were more OTUs in the microbiome of *F. mosseae* and *R. intraradices*
308 hyphosphere than that of *G. margarita* in Exp 1 and Exp 2, respectively (Fig. S4). More
309 importantly, the abundance and structure of over half of the bacteria, at both phyla and
310 genera levels, showed a significant difference between *F. mosseae* and *G. margarita* in
311 Exp 1 and between *R. intraradices* and *G. margarita* in Exp 2 (Fig. 3, 4, 5 and 6). All
312 these results suggested the microbiomes that associated with the three AM fungal
313 species were distinct.
314 Previous studies have indicated that the hyphosphere microbiome are directly
315 involved in soil organic N, P, C mineralization [7,27,31,34,35]. For example,
316 *Pseudomonas* and *Bacillus* are reported to have abilities to mobilize sparingly soluble
317 P in soil (Table S5)[5,7]. In the current study, *G. margarita* harbored greater abundance
318 of *Pseudomonas,* but fewer *Bacillus* than those of *F. mosseae* or *R.intraradices*. In
319 addition, some soil bacteria, called mycorrhiza helper bacteria (MHB), can help AMF
320 colonize more into the root or branch more [9]. MHB belong to many taxa such as
321 Proteobacteria (*Agrobacterium*, *Azospirillum*, *Azotobacter*, *Burkholderia*,
322 *Bradyrhizobium*, *Enterobacter*, *Pseudomonas*, *Klebsiella* and *Rhizobium*), Firmicutes

(*Bacillus*, *Brevibacillus*, and *Paenibacillus*), Actinomycetes (*Rhodococcus*, *Streptomyces*, and *Arthrobacter*) and even some unculturable bacterial taxa such as Acidobacteria (*Acidobacterium*) [36] (Table S6). However, MHB are often AM fungal specificity, which means they can stimulate mycorrhiza formation and extraradical hyphae production for some AMF but inhibit these mycorrhizal traits for the others [37]. For example, *Streptomyces sp*. enhanced the colonization of *R. intraradices* (formerly named *Glomus intraradices*) but inhibited the growth of *Hebeloma cylindrosporum* [38,39]. Here, we found that the abundance of *Streptomyces* and *Bacillus* were much greater in the hyphosphere of *R. intraradices* and *F. mosseae* than that of *G. margarita,* while *G. margarita* which contained the largest abundance of *Pseudomonas*. These observations suggest that different AM fungal species might cooperate with different functional bacteria and have different impacts on the function of the hyphosphere. The COG functional prediction also supported this assertion, indicating that distinct microbiomes recruited by different AM fungi contained different abundance of inorganic P mobilization abilities or other functions (Fig. 7). Further studies are needed to investigate the functions of the hyphosphere microbiome in specific nutrition cycling.

Outlook and conclusion

The soil microbiome is critical to the functioning of plant-AM fungi-bacteria-soil particle continuum system and therefore to growing our food sustainably and with minimal environmental impact, protecting against pathogens and disease, while also providing important ecological services such as nutrient turnover and transformation and bioavailability. Understanding the structure of the microbiome is essential for using the native microbiome efficiently [40]. In recent years, mycorrhizal genome sequencing studies have found that mycorrhizal fungi have lost many saprophytic genes in the long-term co-evolution process with plants [16]. Cooperating with functional microbiomes,

such as phosphatase releasing bacteria [6,41] is considered an important strategy for AM fungi to compensate their lack of ability to utilize organic P. We find for the first time that different living AM fungi species that colonized single plant root recruit active microbiomes which are distinct from each other. The research not only provides direct evidence for understanding the biophysical process that AM fungal hyphae exudates drive the formation of soil bacteria diversity heterogeneity, but also reveals the potential division of labor may exist in plant-AM fungi-bacteria system that still remains to be understood fully. Greater knowledge of these key interactions in the hyphosphere has potential to allow us to more effectively manage the utilization of resources in agricultural systems and help us improve future agricultural sustainability.

Materials and Methods

Soil

A moderately acid soil (Inceptisol according to the USDA classification system) from Tai'an, Shandong province, China (36°10′N, 117°09′E) was used. Physicochemical properties of the soil are presented in Table S1. The collected soil was air dried and sieved (2 mm). The basal nutrients were added to the soil as described in Table S2. The soil was sterilized by gamma irradiation (25 kGy, 60 Co γ-rays) in the Beijing Atomic Energy Research Institute to eliminate indigenous microorganisms and mycorrhizal propagules before use. Previous studies have demonstrated that AM fungi recruited a hyphosphere microbiome that has the potential to stimulate the solubility of organic P [6,7]. In this study, to enhance the colonization of soil microbiome in hyphosphere, 100 mg kg^{-1} myo-inositol hexaphosphate calcium magnesium salt (phytin, TCI, Tokyo, Japan) (equaling to 20 mg P kg^{-1} soil) was added to the hyphal compartment as an organic P resource. In order to induce the AM fungal hyphae to release protons to acidify the hyphosphere soil, $(NH_4)_2SO_4$ was provided as the N

source [42]. In addition, a nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP; 'ENTEC Flüssig' produced by EuroChem Agro GmbH, Mannheim, Germany) was also added, at a rate of 1% (w/w) of the N applied to prevent nitrification of $(NH_4)_2SO_4$.

Microcosms

In order to test whether the extraradical mycelium of each AM fungal species that simultaneously colonized on the same root system would recruit their own microbiome, we used a split-root and compartmented microcosms system that was able to separate the growing spaces of root systems and the extraradical mycelium of two AM fungal species (Fig. 1). The microcosms were constructed using PVC plates, and consisted of four compartments. The two middle compartments were separated by PVC plates and were used for split-root growth (root compartment, RCs). The two outer compartments (hyphal compartment, HCs) were separated from the RCs by a 1 cm buffer zone. The buffer zone consisted of two-layers of 30 μm nylon mesh, which allowed AM fungal hyphae to pass through, but prevented root penetration. In order to easily extract the hyphae, the fine soil used in HCs was sieved with a 30 μm mesh. In short, fine soil was prepared by wet sieving. Approximately 1 kg of air-dried soil was placed into a 5 L bucket, 3-4 L tap water was added, and the soil was brought into suspension by stirring. The soil suspension was poured through a sieve with a mesh width of 30 μm. This procedure was repeated three times on each 1 kg soil portion. The sieved soil suspension was collected in another bucket and allowed to settle until the water above the soil layer became clear and was siphoned off using a flexible tube. The remaining sludge was transferred to a shallow, heat-resistant dish and was dried at 60ºC until the material became solid. The fine soil was then mixed with glass beads (1 mm in diameter) by 1:1 (w/w) ratio. The mixture was sterilized by gamma irradiation (25 kGy, 60 Co γ-rays) in

398 the Beijing Atomic Energy Research Institute. The microcosms received the following
399 amounts of soil or soil-glass bead mixture: 500 g soil in each RC and 165 g soil-glass
400 bead mixture in each buffer zone and 495 g soil-glass bead mixture in each HC. The
401 soil or soil-glass bead mixture was filled very carefully to each compartment to
402 maintain equal bulk density in each HC.

403 **Host plants**

404 Cotton (*Gossypium herbaceum* L., cv. Xinluzao 32) seeds were surface-sterilized
405 with 10% (v/v) H_2O_2 [42], and germinated on moist filter paper for 2 days at 26ºC in
406 the dark. The seeds were then transferred to 40×25 cm moist filter paper for 17 days
407 (12 h light, 12 h dark, 26ºC) to allow the roots to grow longer. Seedlings of similar size
408 and with nine roots (including taproot) were selected, and one plant was transplanted
409 into each microcosm.

410 **AM fungal and bacterial inoculant**

411 The inoculum of *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae*
412 (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A) were purchased from the
413 International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM).
414 They were propagated through hosts (maize: Nongda 108 and *Plantago depressa* Wild.)
415 in zeolite and sand for five months; the spore density was about 20 spores g^{-1} substrate.
416 In order to keep the same RC original microflora, 5 ml of AM fungal inoculum filtrate
417 was added to each RC as described in Table S3. Five ml of soil filtrate was added to the
418 hyphal compartment as the original hyphal compartment microflora. The filtrate of
419 inoculum or soil was obtained by suspending 30 g of unsterilized inoculum or soil in
420 300 ml of sterile water and filtration through six-layer quantitative filter paper
421 (properties similar to Whatman Grade 43) [5], which allowed passing of common soil
422 microbes, but effectively retained spores and hyphae of mycorrhizal fungi.

Experimental design and procedure

Two single factor experiments were conducted, Experiment 1 (Exp 1) and Experiment 2 (Exp 2) which are described in Fig. 1. There were 6 repeats for each treatment, 3 were labeled with $^{13}CO_2$ while the other 3 were given $^{12}CO_2$ treatment as a control. At planting, half of the soil for each RC (250 g) was carefully added to the RC, and then 30 g AM fungal inoculum of each AM fungal species containing about 600 spores was added to each RC. The taproot of the pre-cultured plant was cut-off at the elongation zone, and the shoot was mounted on the central PVC plate. The two groups of lateral roots were evenly separated into the two RCs. Finally, the remaining 250 g soil was added to the RCs. The control treatments (NM) in both experiments received the same amount of sterilized inoculum. The HCs and buffer zone were filled with a soil-glass bead mixture, and thus, the substrates in the four sections are referred to as root soil, buffer soil and hyphal soil (Fig. 1). Plants in these microcosms were grown in a campus greenhouse at China Agricultural University in Beijing from 13 May to 8 July 2015 at 24/30℃ (night/day) and an average photosynthetically actively radiation of 360 μmol m^{-2} s^{-1}. To avoid any possible influence of environmental factors in the glasshouse, the position of the microcosms was re-randomized once a week. Soil gravimetrical moisture was kept at 18-20% (w/w, ~ 70% water holding capacity) with deionized water added to weight every 2 days during the experiment.

$^{13}CO_2$ pulse labeling chamber and procedure

To trace the transfer of plant-derived C from mycorrhizal hyphae to the hyphosphere microbes, $^{13}CO_2$ stable isotope pulse labeling was conducted in the glasshouse for seven days before harvest. Seven weeks after sowing, the cotton plants were subjected to $^{13}CO_2$ (99% of ^{13}C atom) pulse labeling in an airtight Plexiglas growth chamber (Fig. S1). The plant shoots protruded through the holes and the joins

between stems and chamber were sealed with silica gel to prevent direct exposure of the soil surface to the $^{13}CO_2$ labeling. During pulse labeling, a cooling system was used to cool the chamber temperature to 35ºC. A 100 ml aliquot of $^{13}CO_2$ was injected through the septum using a gas-tight syringe every hour from 9 am to 5 pm, the period in which photosynthesis was the greatest during the day [43]. During this process, CO_2 concentration was measured using an infrared gas analyzer. The CO_2 concentration reached about 450 μM after injecting, and about 10 μM before injecting. The lid was removed one hour after the last CO_2 injection, when the $^{13}CO_2$ concentration in the chamber had decreased to atmospheric levels. The plants were labeled for seven days. Simultaneously, the same procedures of $^{12}CO_2$ (99% of ^{12}C atom) labeling control were also performed [5]. To remove the influence of vapor produced by plant evaporation during CO_2 labeling on photosynthesis, three trays of $CaCl_2$ (100 g per tray) were placed in the chamber. The wet $CaCl_2$ trays were removed and dried in a forced-air oven at 105ºC for 2 h every day after the lid of the chamber was removed in the evening and re-used repeatedly.

Harvest and sample analysis

The plants were harvested eight weeks after planting. To prevent contamination of the hyphal samples with exotic bacteria settling on the surface soil, we removed the top 1 cm of soil to reduce any potential contamination. The soil in the buffer zone was removed before collecting the soil from the hyphal compartment. Soil from two HCs of NM treatments was mixed as one sample. A part of the soil was stored at 4ºC for soil tests and another part was immediately frozen in liquid nitrogen and stored at –80ºC until DNA extraction for microbial diversity tests could be performed. The shoots were oven-dried before measuring the dry weight and processing for shoot P concentration. Determination of shoot P concentration was performed according to the method of

Thomas et al. (1967) [44].

Root DNA was extracted using a Tiangen plant genome Kit (Tiangen Co Lt., Beijing, China) following the manufacturer's instructions, the AM fungal gene copies were detected to assess AM fungal root colonization rate. The AMF copies were quantified in triplicate by real-time q-PCR in a q-TOWER q-PCR analyzer (Jena, Germany) using root DNA extracted from each treatment with AM fungi specific primers (Table S4) and using the methods described in supplementary materials. The hyphal length density of HC soil was determined according to the method of Jakobsen et al. (1992) [45].

^{13}C DNA stable isotope probing (SIP) analysis

Soil samples stored at 4ºC were oven-dried at 70ºC, ground, sieved using an 80 µm mesh and then the $\delta^{13}C‰$ was determined at the Stable Isotope Laboratory of the College of Resources and Environmental Sciences, China Agricultural University, Beijing, China (see details in supplementary materials). These soils were assumed to only contain ^{13}C contained in AM fungal extraradical hyphae or released by the hyphae to the soil.

Collection of extraradical mycelia from hyphal compartment

Five hundred g of the soil, glass beads, and associated fungal material in the hyphal compartments were transferred to a sieve with a 30 µm mesh. The soil was carefully washed through the mesh with filtered sterile deionized water, leaving the extraradical mycelium and glass beads on the sieve. To separate the extraradical mycelia from the glass beads and to clean them, the mixture was transferred into a 1 L beaker and filtered sterile deionized water was added, before the mixture was stirred and poured back into the sieve, leaving the glass beads in the beaker. This procedure was repeated five times. The extraradical mycelia were rinsed with filtered sterile deionized water before they

were collected from the sieve using forceps and placed into a microcentrifuge tube. All mycelia samples were weighed before DNA extraction and afterwards stored at −80°C until further processing.

For the non-mycorrhizal treatments, no extraradical hyphae were observed in the hyphal compartment when samples were collected as described above. 0.5 g residual soil particles on the sieve were collected and referred to as non-mycorrhizal samples. These samples were also stored at −80°C before DNA extraction and tagged as NM (non-mycorrhizal treatment).

DNA extraction, density gradient centrifugation and q-PCR analysis

DNA of AM fungal mycelia and soil sample collected from last step were extracted using the FastDNA SPIN Kit (MP Biomedicals LLC, Santa Ana, CA, USA) following the manufacturer's instructions. All the extracted rDNA samples (approximately 500 ng) were fully blended with cesium trifluoroacetate (CsTFA) to achieve an initial buoyant density (BD) of 1.560 g ml^{-1} before ultracentrifugation at 45400 rpm for 36 h [46]. The centrifuged gradients were fractionated from bottom to top into 16 equal fractions. The buoyant density of DNA in the gradient fractions was determined using a digital refractometer (Reichert AR2000). The DNA fractions were then purified with isopropyl alcohol and 70% (v/v) ethanol and stored at –80ºC for further analysis. DNA from each gradient fraction of all treatments was quantified in triplicate by real-time q-PCR in an q-TOWER q-PCR analyzer (Jena, Germany) with primers Ba519f/Ba907r (Table S4) using the protocol described in the supplementary material.

16S rRNA gene-based Miseq sequencing

Fractions which had buoyant density of approximately 1.58 were quality checked, and then the DNA samples were sent to the Majorbio Biotechnology Company (Shanghai, China) for sequencing on an Illumina MiSeq (PE300) sequencing platform.

The V3–V4 hypervariable regions of 16S rDNA were amplified using a primer set Ba338f/Ba806r (Table S4). The DNA samples from the NM control soil were sent for sequencing and used as the original soil microbiome community. The DNA samples of AM fungal mycelia were considered as hyphosphere microbiome. In addition, ^{12}C labeled samples were sequenced and used as the whole hyphosphere microbiome, while ^{13}C labeled samples were used as the active hyphosphere microbiome which influenced by hyphal exudates directly.

Processing of sequencing data

The Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) pipeline was used to process the sequencing data, as described previously [47]. The raw sequencing reads were identified to operational taxonomic units (OTUs) according to the methods in supplementary material. The sequences obtained in this study were deposited in the GenBank database under accession number PRJNA556534.

Statistical analysis

Split-root experiment data from two experiments were analyzed separately. Data from *F.m* and *G.m* HCs in Exp 1 or *R.i* and *G.m* HC in Exp 2 were compared to determine the difference between different AM fungal species. Data from non-mycorrhiza (NM) control was also compared with *F.m* and *G.m* in Exp 1 or *R.i* and *G.m* in Exp 2 to determine the effect of AM fungal inoculation. Before ANOVA, AM fungi DNA copy number was used to assess mycorrhizal colonization rate and log-10 transformed. Likewise, the data for the relative abundance of ^{13}C in HC soil, taxa groups (phyla and genera) and Cluster of Ortholog Genes (COG) functional pathways were arcsine- transformed. SPSS 21.0 (Statistical Product and Service Solutions, IBM, the USA) was employed to conduct above analysis.

Shoot biomass, P concentration and content data were analyzed separately for the

NM or AM (*F.m*/*G.m* or *R.i*/*G.m*) as the treatment factor. A *posteriori* comparison was made using Turkey tests *(P*<0.05) by SPSS v16.0. SPSS 21.0 (Statistical Product and Service Solutions, IBM, the USA) was employed to conduct above analysis.

The rarefaction curve of OTUs for each treatment was calculated by Usearch (version 7.0, http://drive5.com /uparse/). The sequencing results of the $^{13}CO_2$ pulse labeling samples were used to stand for the active hyphosphere microbial community.

Bray-curtis distances of 16S rRNA genes in nonmetric Principal Component Analysis (PCA) was calculated by QIIME software, then analyzed by vegan package in R (v 2.4.2) to compare the β-diversity of each experiment. Significance of the data was estimated using Adonis with *P*<0.05 by vegan package in R (v 2.4.2).

The OTUs of 16S rDNA were standardized by PICRUSt (PICRUSt software stores COG information corresponding to Greengene id), and the COG family information corresponding to each OTU through Greengene id corresponding to each OTU for functional prediction obtained.

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Additional file

Real time q-PCR analysis protocol;

^{13}C DNA stable isotope probing (SIP) analysis;

Processing of pyrosequencing data;

Fig. S1 Root growth system and $^{13}CO_2$ isotope probing equipment;

Fig. S2 Photograph before harvesting;

Fig. S3 Quantitative distribution of density-resolved bacterial 16S rRNA genes obtained from hyphospheres of different inoculation treatments after a 7-day labeling period with $^{13}CO_2$ and $^{12}CO_2$;

Fig. S4 Rarefaction curves of the sequences;

Fig. S5 Taxonomic assignment of sequence data at the phylum level;

Fig. S6 The Principal Component Analysis (PCA) of 16S rDNA from all 30 samples;

Table S1. The physicochemical properties of the soil used in this study;

Table S2. Basal mineral nutrients added to the soil;

Table S3. Arbuscular mycorrhizal inoculation treatments and inoculum filtrates supplied to RCs;

Table S4. Details of primers used in this experiment;

Table S5. The relative abundance (%) of phosphate solubilizing bacteria (PSB) referred to previous studies;

Table S6. The relative abundance (%) of mycorrhizal helper bacteria (MHB) referred to previous studies;

Reference

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Not applicable

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598 Scottish Government.

599 **Availability of data and materials**

600 The Illumina MiSeq sequence datasets are available at the NCBI Sequence Read
601 Archive BioProject ID PRJN556534.

602 **Authors' contributions**

603 G Feng, XF Chai and JC Zhou designed the study. JC Zhou and XF Chai performed
604 the plant culture and chemical analysis. JC Zhou prepared samples for amplicon
605 sequencing and performed bioinformatics and statistical analyses. JC Zhou, L Zhang,
606 G Feng, TS George contributed to statistical interpretation of results and wrote the
607 manuscript. All authors read and approved the final manuscript.

608 **Ethics approval and consent to participate**

609 Not applicable.

610 **Consent for publication**

611 Not applicable.

612 **Competing interests**

613 The authors declare that they have no competing interests.

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Figure and table legend

Fig. 1 The experimental design and plant growth system. The host plant was cotton *(Gossypium herbaceum L.)*. RC and HC denote the root compartment and hyphal compartment, respectively. The dotted lines indicate a 30 μm nylon mesh and the zone between two meshes was a buffer zone. Exp 1 and Exp 2 refer to two independent experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different AM fungal inocula. St means sterilized. The information on AM fungal inoculation treatments and inoculum filtrates supplied

to RCs is shown in the table S3. The brown circles represent the original bacterial community from soil. The red, blue and purple lines represent the hyphae of *F.m*, *G.m* and *R.i*, respectively. While the red, blue and purple circles represent the original bacterial community from *F.m*, *G.m* and *R.i* inoculum, respectively.

Fig. 2 The log AM fungal DNA copy number in the roots of cotton plants (a); hyphal length density (b) and ^{13}C abundance of the HC soil (c). Exp 1 and Exp 2 refer to the two independent experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different AM fungal inocula. All the treatments showed in this part were ^{13}C labeled. **: $P<0.01$, ***: $P<0.001$.

Fig. 3 Phylum level distribution of DNA sequences. Exp 1 and Exp 2 refer to two independent experiments. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments shown were ^{13}C labeled. *, ** and ** mean this phylum was in greater abundance in this condition in same experiment in $P<0.05$, 0.01 or 0.001 level, respectively.

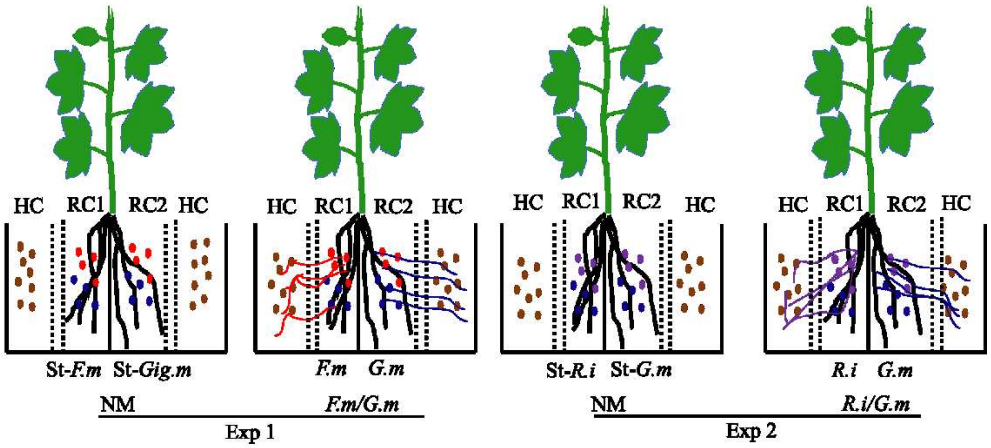
Fig. 4 Genus level distribution of DNA sequences. The genus showed in this plot were dominant, which occupied over 1%, others were summed in Others. Exp 1 and Exp 2 refer to two independent experiments. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments shown in this part were ^{13}C labeled. *, ** and ** mean this genus was in greater abundance in this condition in same experiment in $P<0.05$, 0.01 or 0.001 level, respectively. The red named genus occurred in both AM fungal hyphosphere of same experiment.

Fig. 5 Venn plot of number and proportion of genera in *F.m*/*G.m* of Exp 1 and *R.i*/*G.m* of Exp 2. The overlapping area refers to the genera without a significant difference in relative abundance among different inoculation treatments (^{13}C samples), while the number and percentage in brackets means the percentage of genera with a significant difference in relative abundance among different inoculation treatments (^{13}C samples). The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments showed in this part were ^{13}C labeled.

Fig. 6 The Principal Component Analysis (PCA) of 16S rRNA genes in Exp 1 (a) and Exp 2 (b). Exp 1 and Exp 2 refer to two independent experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different AM fungal inocula. The *P* value in this part was analyzed by permutational MANOVA (eg. adonis).

Fig. 7 Heatmap plot of Cluster of Ortholog Genes (COG) functional pathway relative abundance under in *F.m*/*G.m* of Exp 1 and *R.i*/*G.m* of Exp 2. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments showed in this part were ^{13}C labeled. Different letters mean significant difference between different treatments at $P<0.05$ level.

Table 1 Biomass, Phosphorus (P) concentration and P content of shoots in different inoculation treatments.



824

825 **Fig. 1** The experimental design and plant growth system. The host plant was cotton
826 *(Gossypium herbaceum L.)*. RC and HC denote the root compartment and hyphal
827 compartment, respectively. The dotted lines indicate a 30 μm nylon mesh and the zone
828 between two meshes was a buffer zone. Exp 1 and Exp 2 refer to two independent
829 experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus*
830 *intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora*
831 *margarita* (*G.m*) (JA101A), the three different AM fungal inocula. St means sterilized.
832 The information on AM fungal inoculation treatments and inoculum filtrates supplied
833 to RCs is shown in the table S3. The brown circles represent the original bacterial
834 community from soil. The red, blue and purple lines represent the hyphae of *F.m*, *G.m*
835 and *R.i*, respectively. While the red, blue and purple circles represent the original
836 bacterial community from *F.m*, *G.m* and *R.i* inoculum, respectively.

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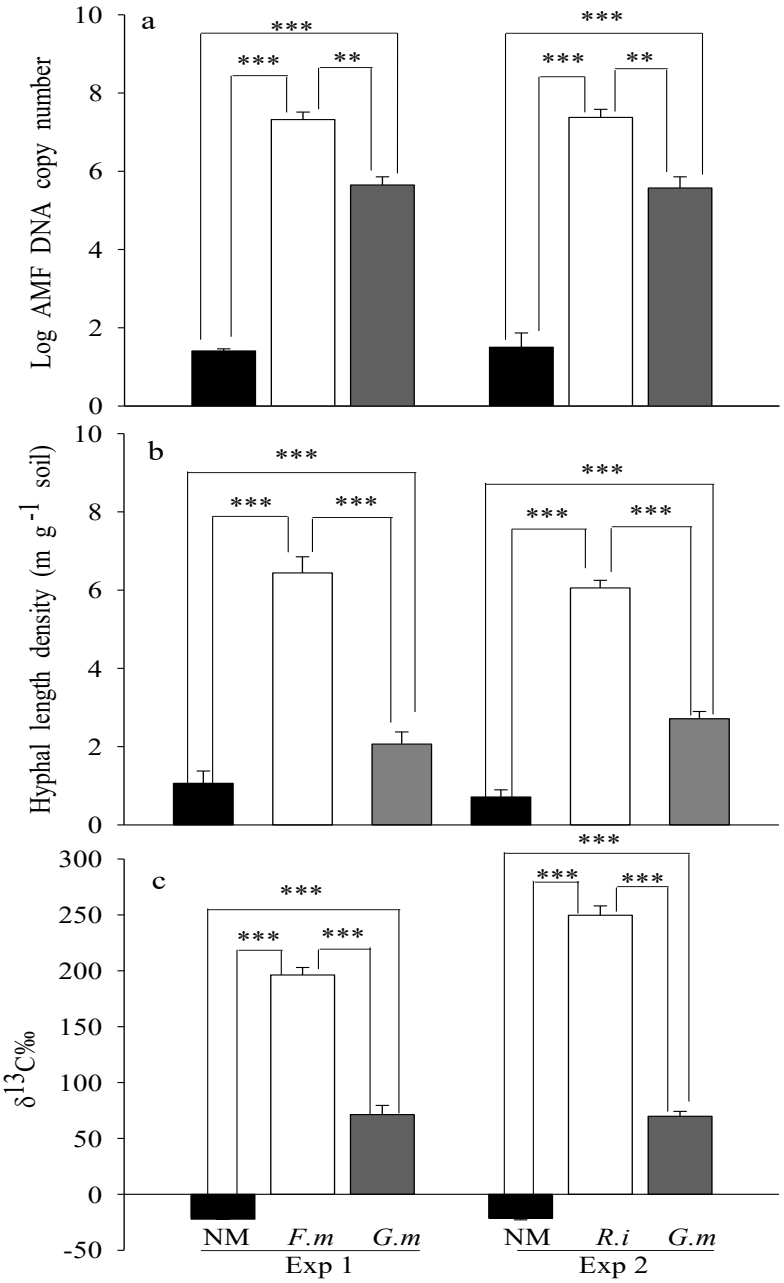


Fig. 2 The log AM fungal DNA copy number in the roots of cotton plants (a); hyphal length density (b) and ^{13}C abundance of the HC soil (c). Exp 1 and Exp 2 refer to the two independent experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different AM fungal inocula. All the treatments showed in this part were ^{13}C labeled. **: $P<0.01$, ***: $P<0.001$.

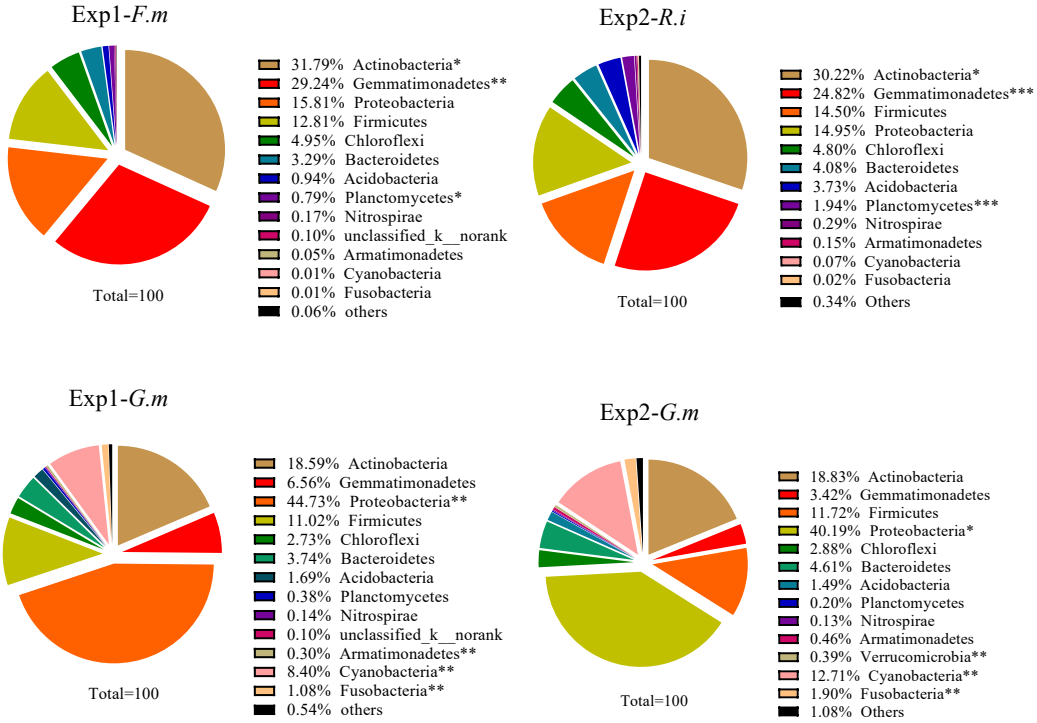


Fig. 3 Phylum level distribution of DNA sequences. Exp 1 and Exp 2 refer to two independent experiments. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments shown were ^{13}C labeled. *, ** and ** mean this phylum was in greater abundance in this condition in same experiment in $P<0.05$, 0.01 or 0.001 level, respectively.

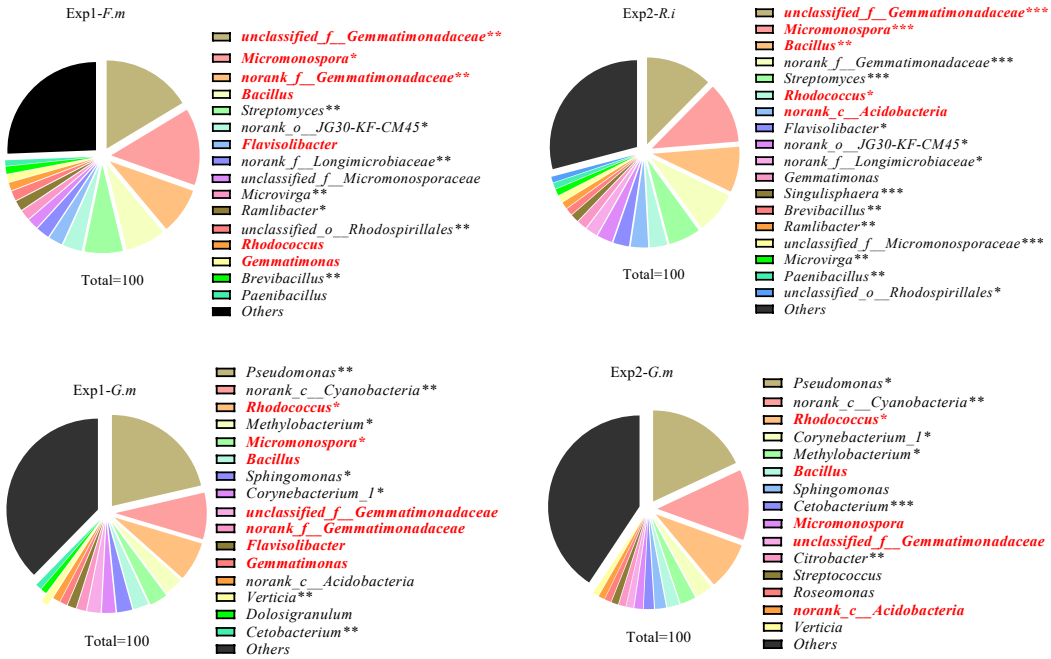
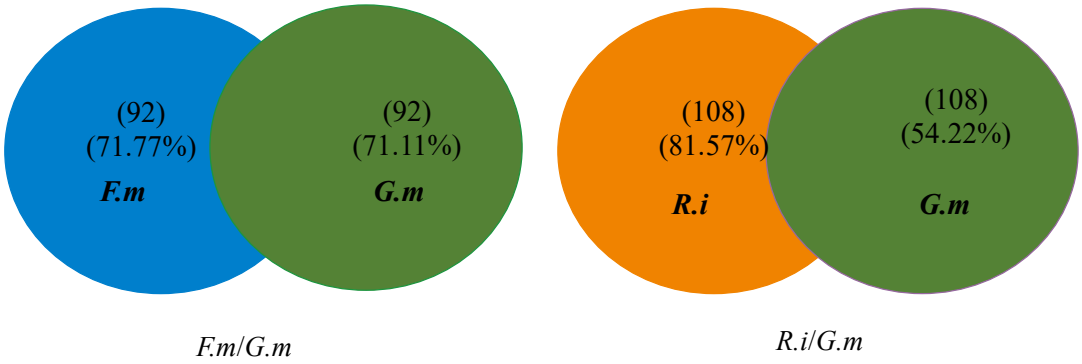


Fig. 4 Genus level distribution of DNA sequences. The genus showed in this plot were dominant, which occupied over 1%, others were summed in Others. Exp 1 and Exp 2 refer to two independent experiments. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments shown in this part were ^{13}C labeled. *, ** and ** mean this genus was in greater abundance in this condition in same experiment in $P<0.05$, 0.01 or 0.001 level, respectively. The red named genus occurred in both AM fungal hyphosphere of same experiment.



F.m/*G.m* *R.i*/*G.m*

Fig. 5 Venn plot of number and proportion of genera in *F.m*/*G.m* of Exp 1 and *R.i*/*G.m* of Exp 2. The overlapping area refers to the genera without a significant difference in relative abundance among different inoculation treatments (^{13}C samples), while the number and percentage in brackets means the percentage of genera with a significant difference in relative abundance among different inoculation treatments (^{13}C samples). The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments showed in this part were ^{13}C labeled.

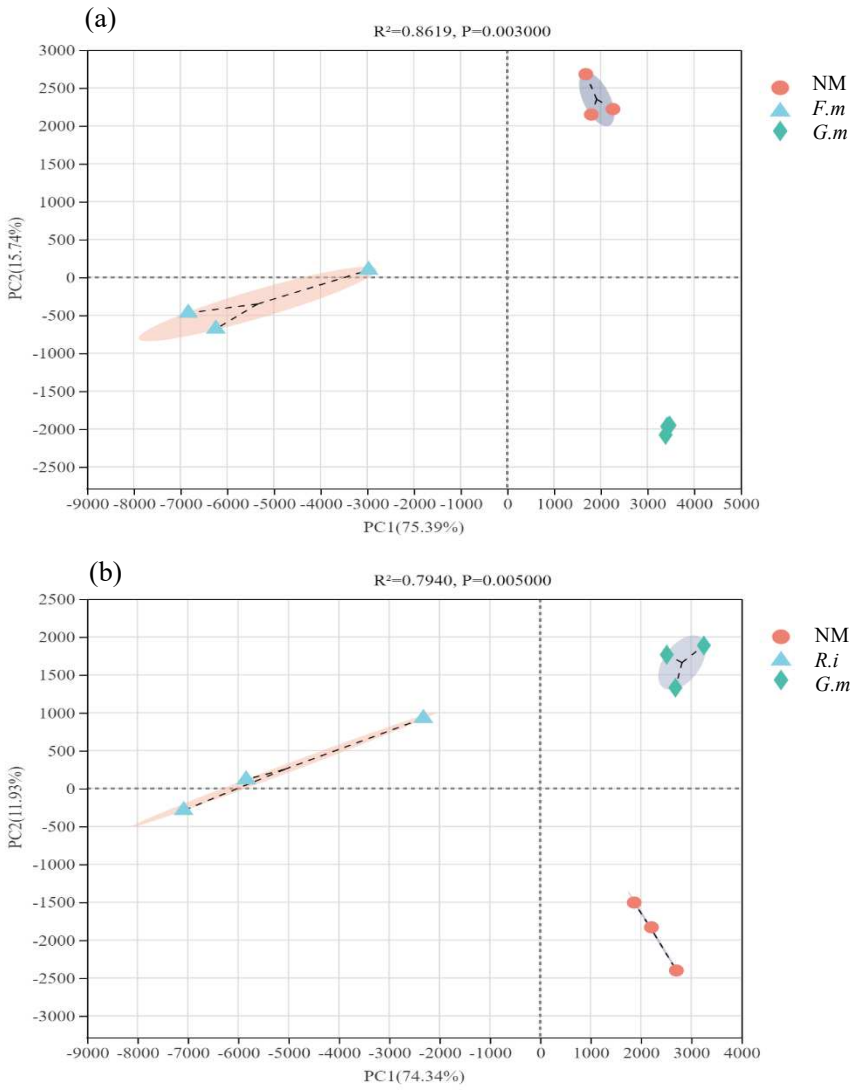


Fig. 6 The Principal Component Analysis (PCA) of 16S rRNA genes in Exp 1 (a) and Exp 2 (b). Exp 1 and Exp 2 refer to two independent experiments. The nonmycorrhizal (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different AM fungal inocula. The *P* value in this part was analyzed by permutational MANOVA (eg. adonis).

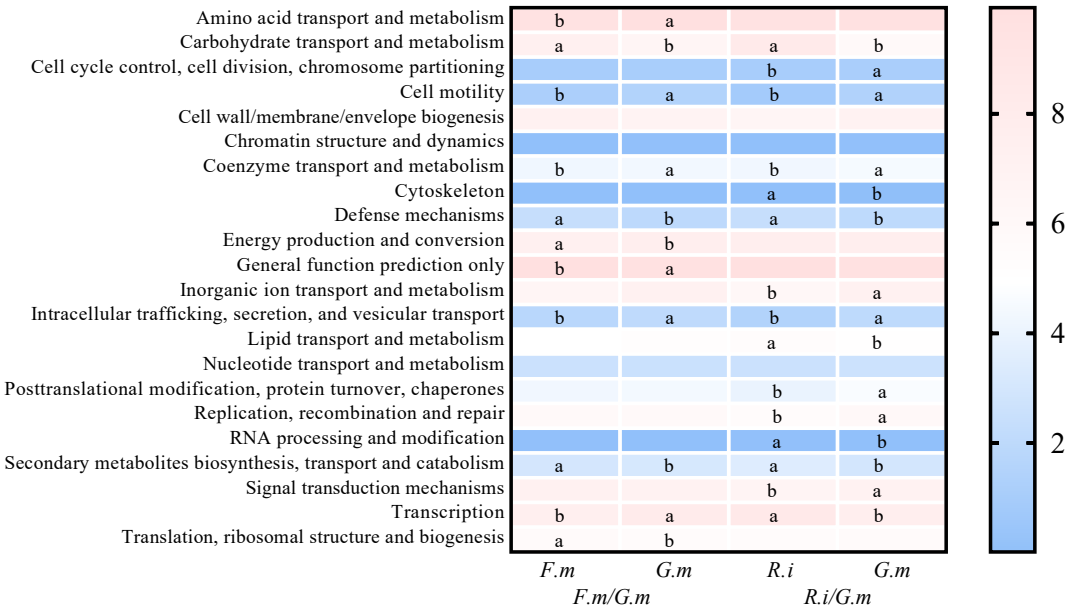


Fig. 7 Heatmap plot of Cluster of Ortholog Genes (COG) functional pathway relative abundance under in *F.m*/*G.m* of Exp 1 and *R.i*/*G.m* of Exp 2. The three different AM fungal inocula were *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A). All the treatments showed in this part were ^{13}C labeled. Different letters mean significant difference between different treatments at $P<0.05$ level.

957 **Table 1** Biomass, Phosphorus (P) concentration and P content of shoots in

958 different inoculation treatments.

Conditions		Biomass(g)	P concentration (mg g^{-1})	P content (mg $plant^{-1}$)
Exp 1	NM	0.82±0.06	0.91±0.04	0.74±0.05
	F.m/*G.m*	5.93±0.25 **	1.49±0.06 *	8.84±0.47 ***
Exp 2	NM	0.79±0.06	0.87±0.07	0.67±0.04
	R.i/*G.m*	5.78±0.30 **	1.92±0.09 **	11.03±0.59 ***

959 Note: Exp 1 and Exp 2 refers to two independent experiments. The nonmycorrhizal

960 (NM) control is compared to *Rhizophagus intraradices* (*R.i*) (EY108), *Funneliformis*

961 *mosseae* (*F.m*) (MD118) and *Gigaspora margarita* (*G.m*) (JA101A), the three different

962 AM fungal inocula. All the treatments shown in this part were ^{13}C labeled. The values

963 in the tables were mean value, and *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

Figures

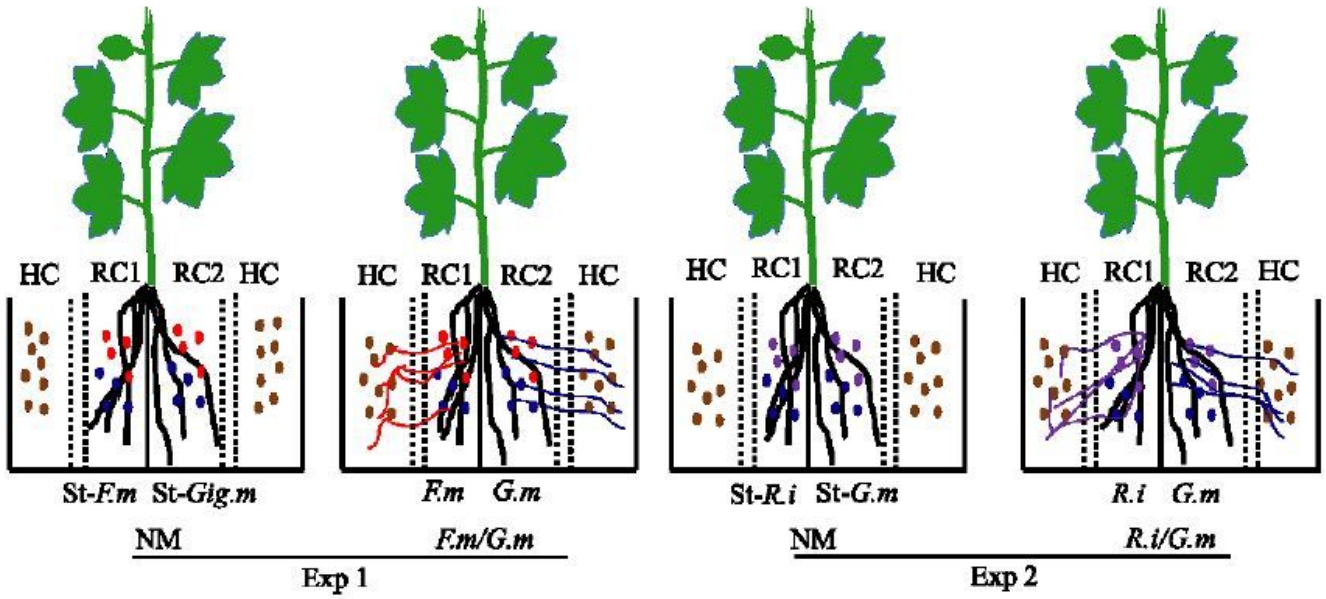


Figure 1

Figure 1

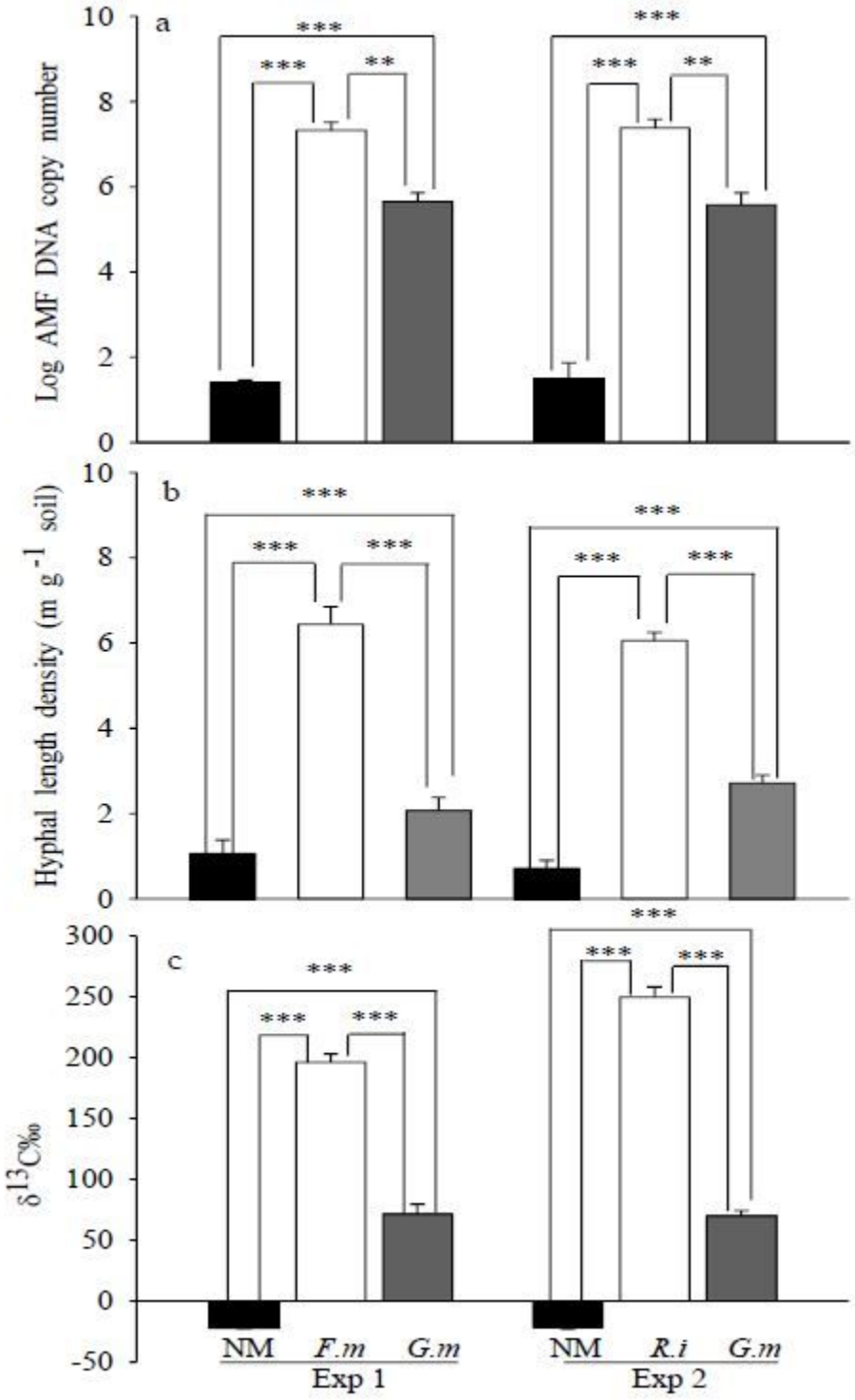


Figure 2

Figure 2

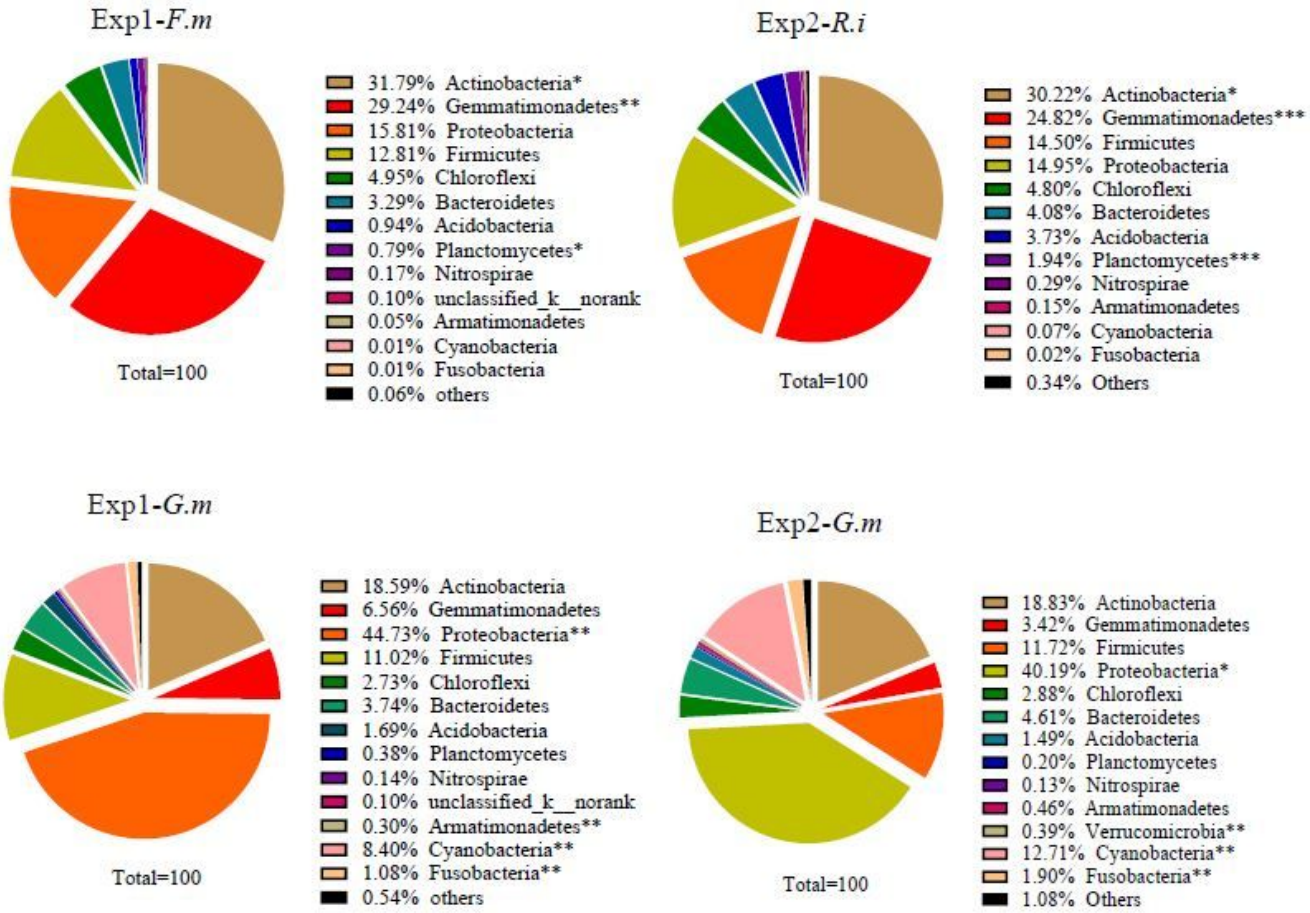


Figure 3

Figure 3

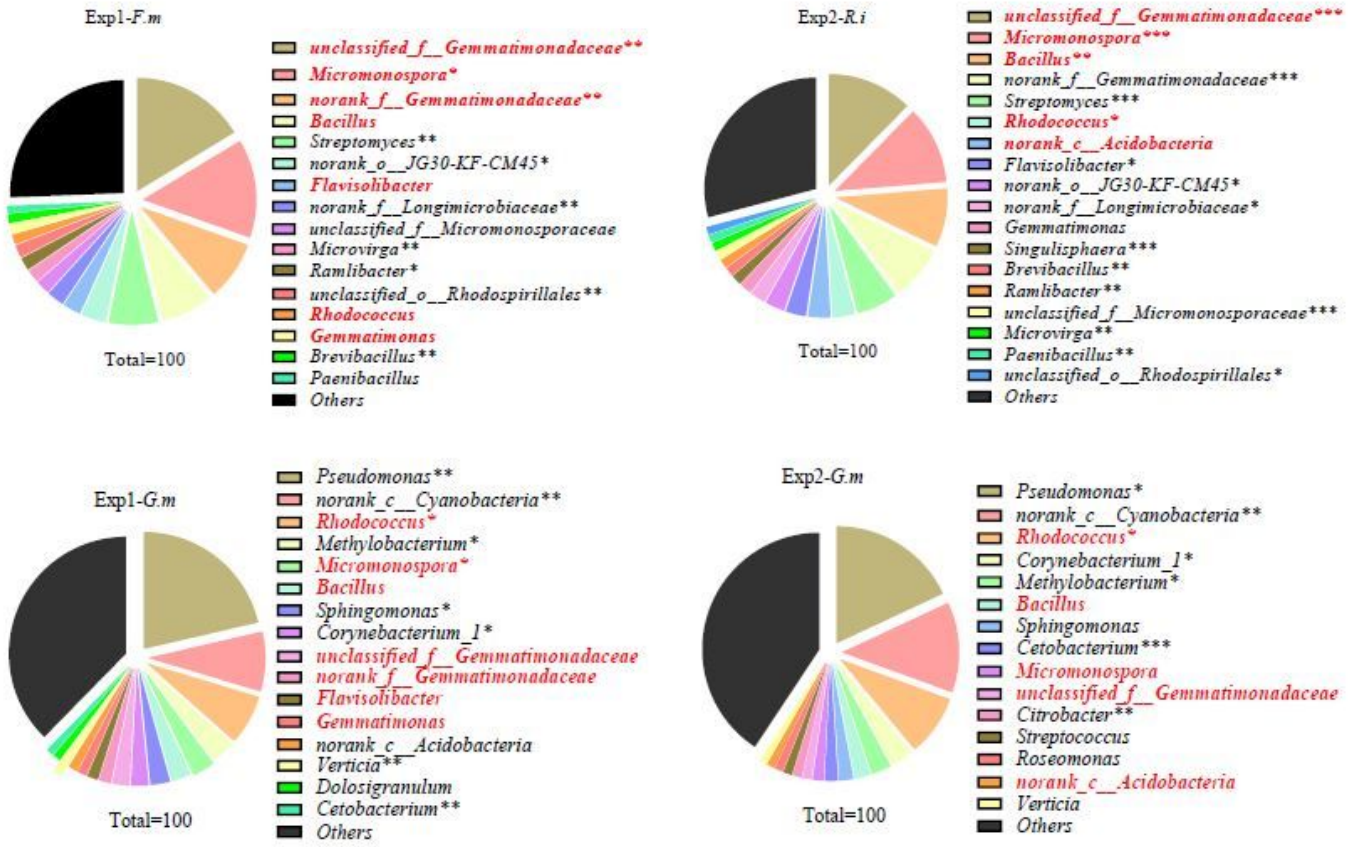


Figure 4

Figure 4

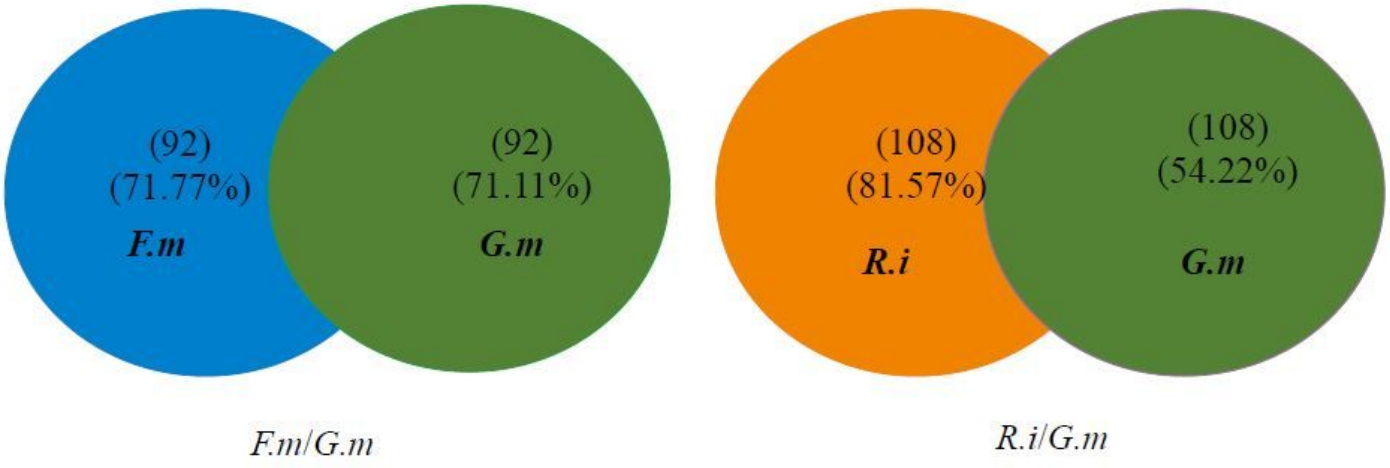


Figure 5

Figure 5

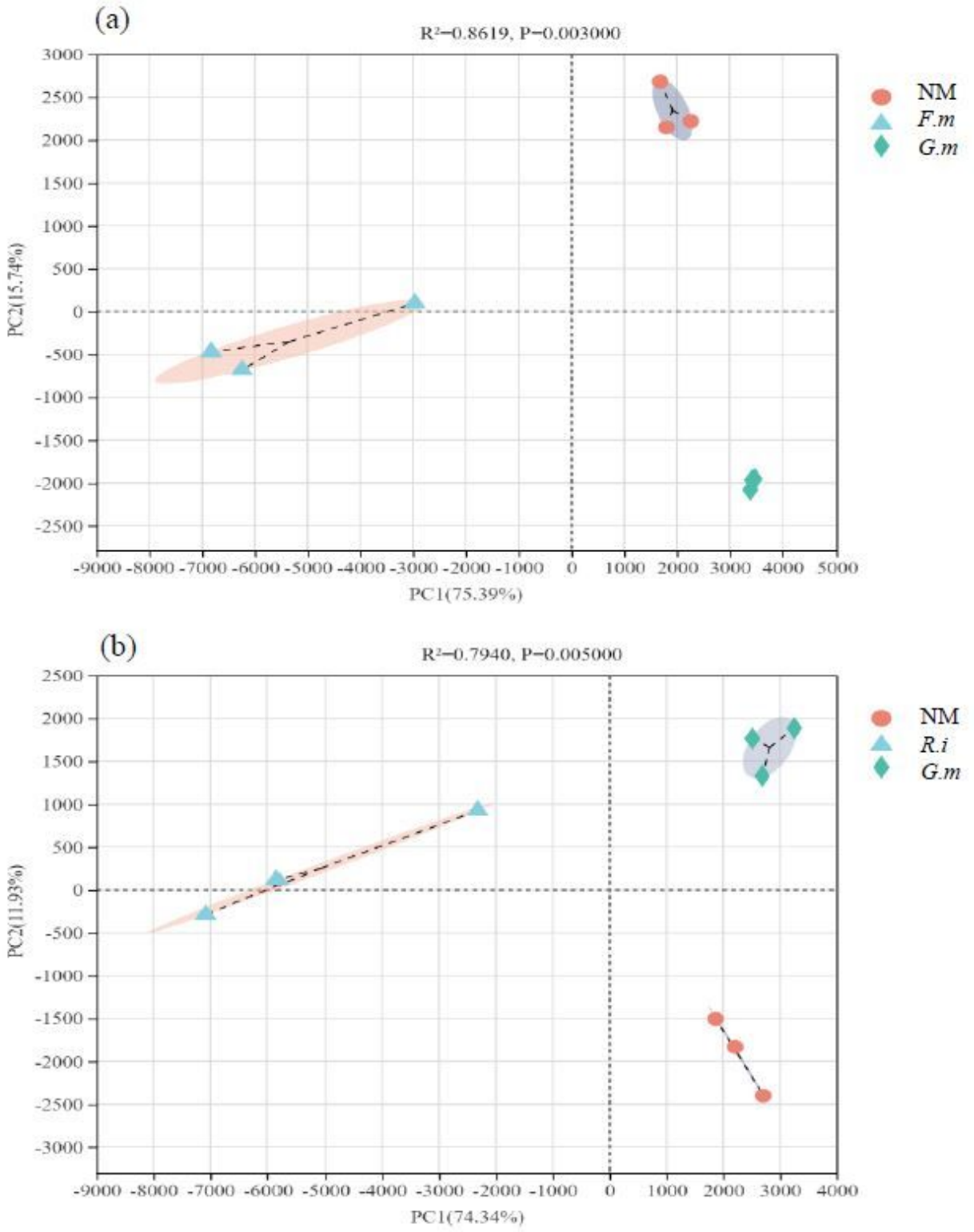


Figure 6

Figure 6

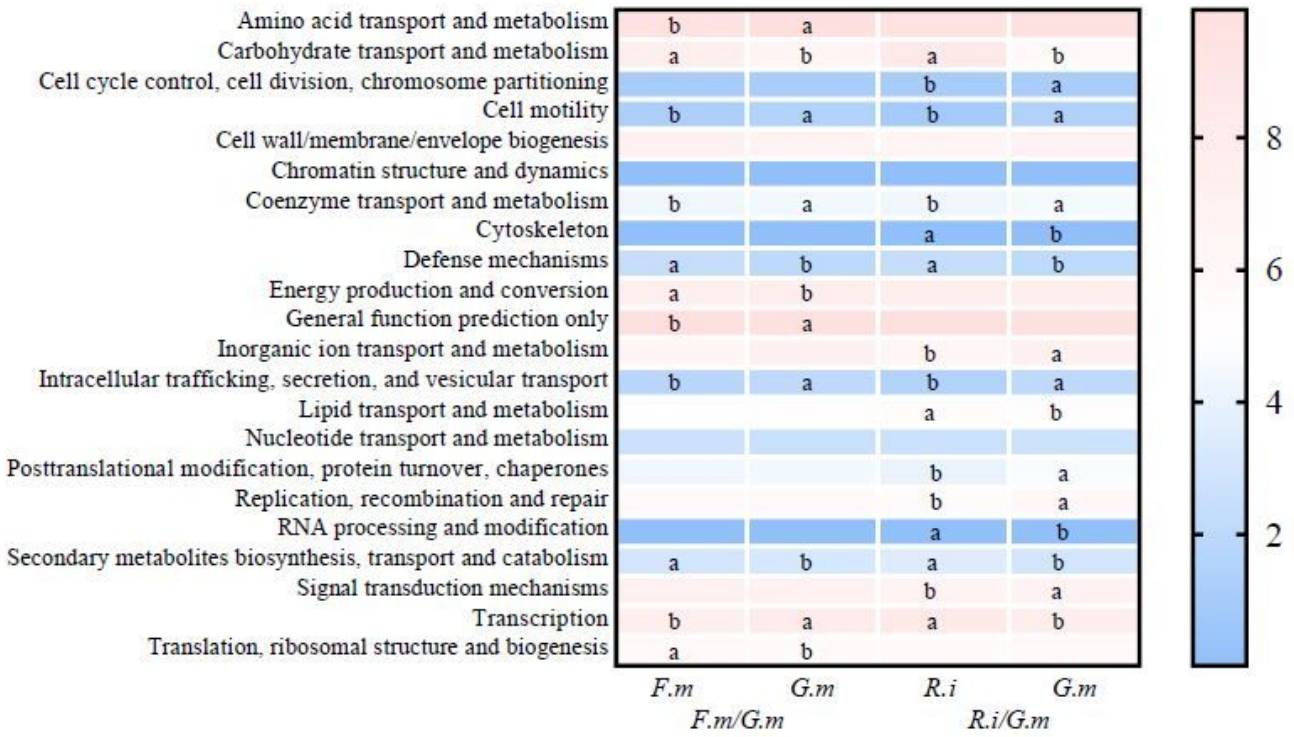


Figure 7

Figure 7

Supplementary Files

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- Supplementaryimformation.docx