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RESEARCH

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Metabolomic approach reveals the mechanism of synthetic communities to promote high quality and high yield of medicinal plants—danshen (*Salvia miltiorrhiza* Bge.)

Hong-Mei Jia[1,2†], Chang-Wen Zheng[1,2†], Yu-Rui Wu[4], Hai Wang[3*] and Zhu-Yun Yan[1,2*]

Abstract

Background *Salvia miltiorrhiza* Bunge, a significant and widely used medicinal herb, is also recognized in the US Pharmacopoeia as a dietary supplement. However, the decline in yield and quality limits its further development as a traditional herbal medicine. Therefore, a deeper understanding of how synthetic communities (SynCom) affect the quality and yield of *S. miltiorrhiza* and the underlying mechanisms is necessary.

Results In this study, we selected *S. miltiorrhiza* as the research subject and designed two synthetic communities (SynCom 1 and SynCom 2) using five endophytic fungi without significantly growth-promoting effect. We conducted both greenhouse and field experiments to investigate their impact on the yield and quality of the herbal plants. Greenhouse experiments confirmed that SynCom 1 significantly increased the biomass of *S. miltiorrhiza*, whereas SynCom 2 had the opposite effect. Field experiments further demonstrated that the application of SynCom 1 promoted photosynthesis and enhanced carbon and nitrogen metabolism, steady and markedly promoted plant growth, and thus increased *S. miltiorrhiza* yield compared to the uninoculated. In contrast, SynCom 2 inhibited yield but increased the content of the main active components. Un-targeted metabolomics analysis showed that SynCom 1 mainly promoted tricarboxylic acid cycle and nitrogen assimilation process to increase yield, and SynCom 2 mainly increase substrate content in the salvianolic acid and tanshinone synthesis pathways to improve quality.

Conclusion These beneficial qualities exhibited by SynComs composed of fungi without apparent growth-promoting abilities represent an untapped resource that can be leveraged to enhance crop productivity. This opens up new research avenues for precision manipulation of plant microbiomes.

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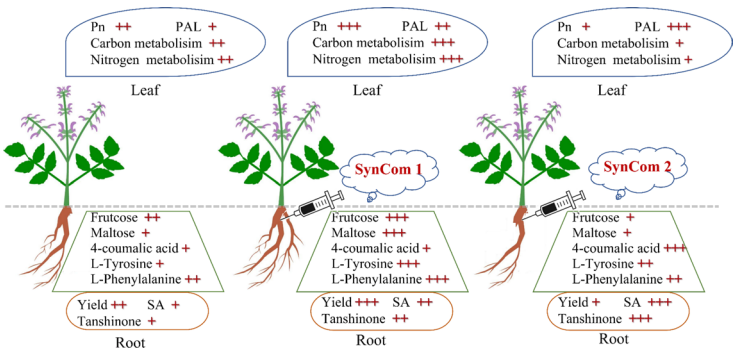
Highlights

1 The Synthetic communities with non-growth promoting fungi promote the growth and quality of Salvia miltiorrhiza Bge.
2 SynCom 1 promoted tricarboxylic acid cycle and nitrogen assimilation process to increase yield.
3 SynCom 2 increase substrate content in the salvianolic acid and tanshinone synthesis pathways to improve quality.
4 The SynComs possess the potential to be applied to field planting and promote medicine food homology plant development as well.
5 The addition of distant species changes the interaction pattern among closely related species.

Keywords SynComs, Quality, Microbiomes, Medicinal plants, *Salvia miltiorrhiza* Bge

Graphical abstract



Introduction

Medicinal plants are widely used in the prevention of diseases [17]; however, the decrease in medicinal ingredients and yield greatly restrict their application [36]. Previous studies have characterized that a single microorganism positively impacts the yield and quality of herbal medicine [15, 34]. Endophytic fungi play a growth-promoting role by producing plant hormones, strengthening photosynthetic capacity, and enhancing carbon and nitrogen metabolism [7, 9, 10, 18]. Meanwhile, fungi can also impact herbal medicine quality by affecting the biosynthesis and accrual of secondary metabolites. However, there is now general consensus that utilizing a single strain to improve growth and development is often limited and unstable [4].

Under natural conditions, the interactions between microbial communities and plants are complex, complicating the elucidation of the functions and mechanisms underlying the reciprocal relationships between specific strains and herbal medicines. This complexity also hinders the practical application of microbial communities in production settings. Therefore, designing synthetic communities (SynComs) is essential to simplify the study of these intricate interactions [27]. Recently, the development and construction of SynComs has provided functional and mechanistic insights into the magnitude of plant-related microbial communities for plant growth and development. Accordingly, it is a feasible method to increase plant yield and active component content using SynComs. Unlike conventional crop cultivation, which typically prioritizes yield, the quality of herbal medicines is a critical factor that cannot be overlooked. Therefore, optimizing SynComs to enhance both quality and yield consistently is crucial. Currently, the construction of SynComs predominantly relies on selecting microorganisms with potential growth-promoting functions [11, 28]. However, there is limited knowledge on whether SynComs constructed from endophytic fungi, which lack obvious growth-promoting functions, can positively impact medicinal plants.

Current research on the functions of SynComs is primarily conducted under controlled conditions, which do not always translate directly to practical benefits in the field. There is limited information on whether the

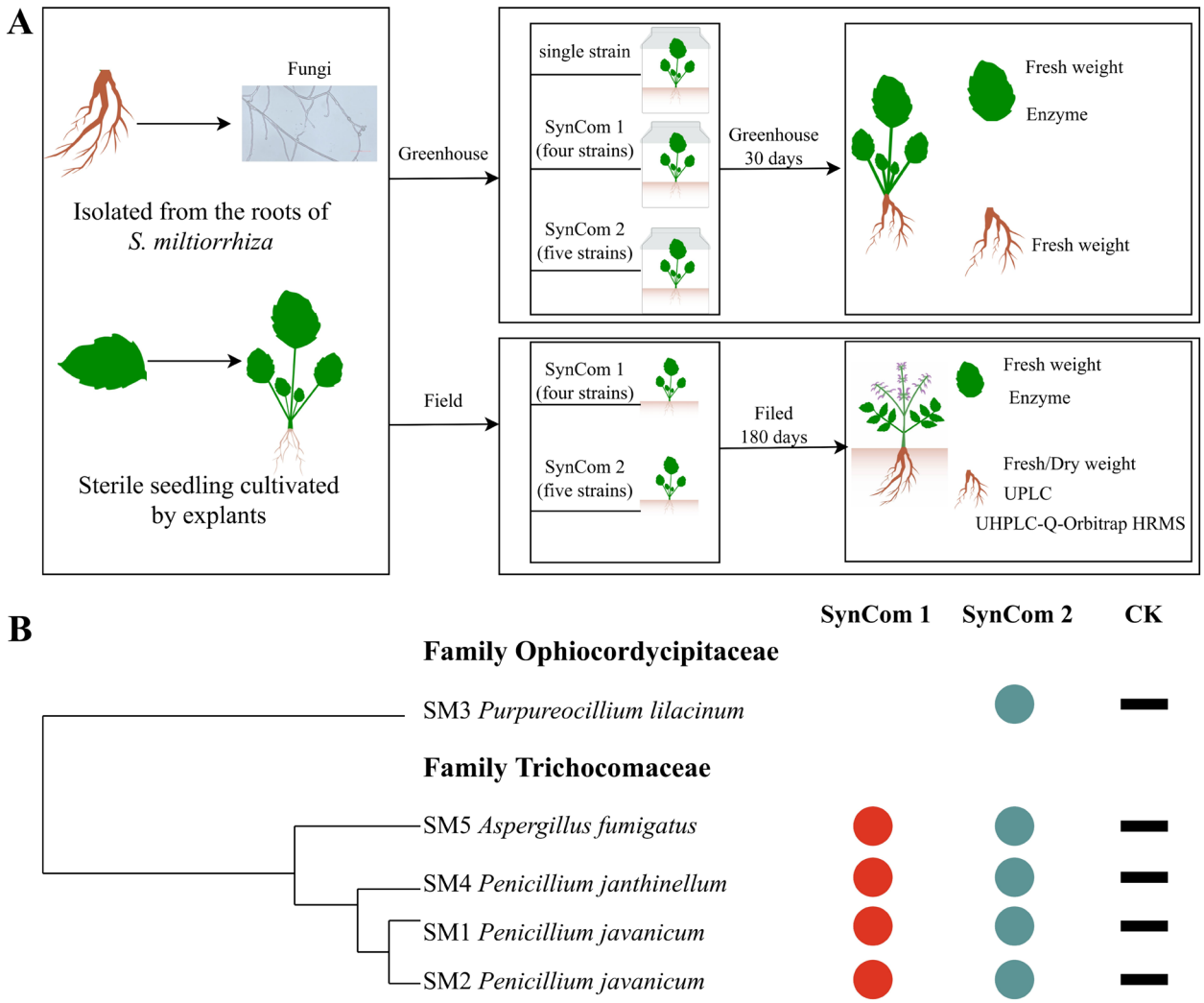


Fig. 1 The experimental process and design of endophytic fungi inoculation treatment. CK is the control group with no fungi. **A** The experimental process. Single strain means any one of the five strains of fungus. **B** Design of endophytic fungi inoculation treatment

comprehensive effects of SynComs on herbal medicine can achieve the expected stabilizing outcomes under complex field environments. Therefore, investigating the effectiveness of SynComs on the yield and active components of herbal medicine in field environments is of great significance for the cultivation and production of medicinal plants.

Salvia miltiorrhiza Bunge is one of the most important traditional Chinese herbs in China. In addition, *S. miltiorrhiza.* is supplied as tea, beverages, soup, and dietary supplements. Tanshinones (such as tanshinone IIA (T-IIA), dihydrotanshinone (DT), and miltrione (MT)) and salvianolic acid (such as rosmarinic acid (RA), salvianolic acid B (SAB), and salvianolic acid A (SAA)) are specialized and active metabolites in the root and rhizome of *S. miltiorrhiza*. However, the yield and quality of *S. miltiorrhiza* decreased due to years of cultivation [3, 35]. Research has indicated that utilizing a single strain can enhance the production of tanshinones or salvianolic acids [1, 20, 22]. However, there are limited reports on the impact of synthetic communities on the levels of active ingredients in *S. miltiorrhiza*. For this study, *S. miltiorrhiza.* was selected as the experimental subject. Initially, we investigated the effects of five endophytic fungi on the primary metabolism of tissue culture seedlings. Five endophytic fungi that did not exhibit significant growth-promoting effects were subsequently used to construct two different synthetic communities (SynComs). Both greenhouse and field experiments were conducted to examine the effects of these SynComs on enzyme activities, biomass, and the content of the main active components of *S. miltiorrhiza.* Finally, untargeted metabolomics was employed to analyze the regulatory

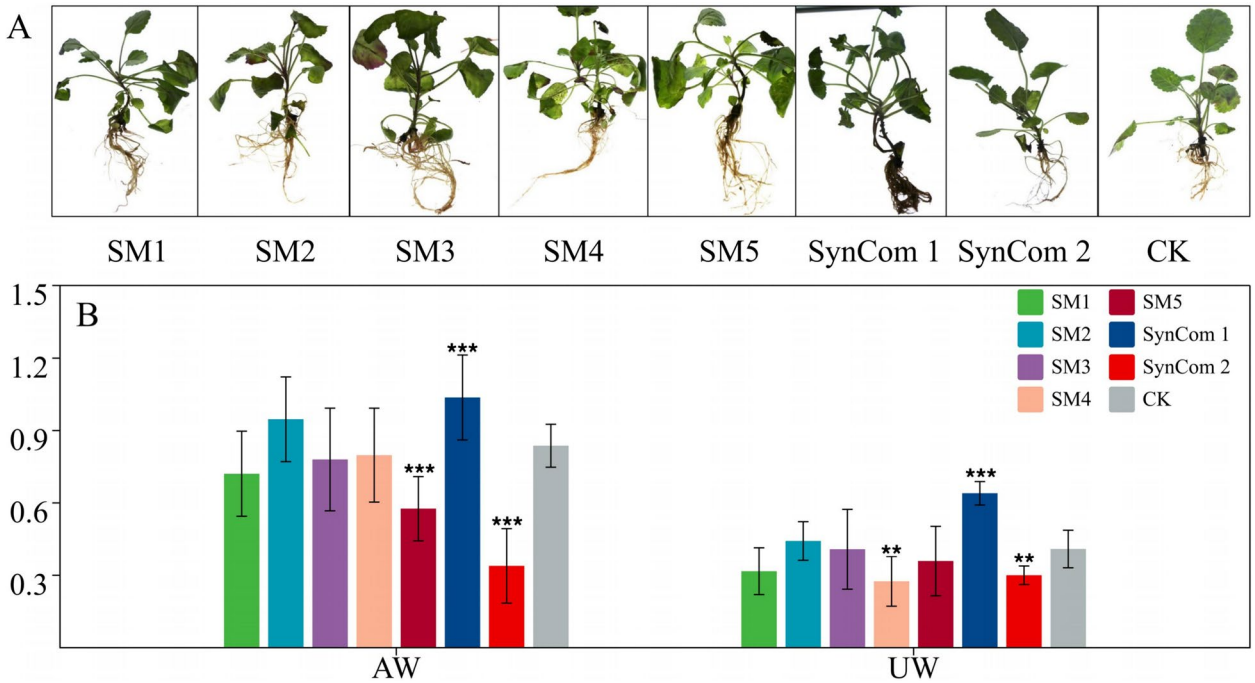


Fig. 2 Effects of endophytic fungi inoculation on the biomass of *S. miltiorrhiza*. **A** The growth of greenhouse seedlings. **B** AW and UW biomass. *p value < 0.05, **p value < 0.01, ***p value < 0.001. *AW* the fresh weight of the shoot, *UW* the fresh weight of the root

mechanisms of different SynComs on the yield and quality of *S. miltiorrhiza*.

Our study demonstrated that constructing SynComs with strains that do not significantly promote growth can substantially enhance plant quality and yield. This suggests that the growth-promoting effect of a strain should not be the sole criterion in SynCom design. Our findings provide a basis for the expanded application of non-growth-promoting microorganisms. These SynComs represent promising resources for advancing microbial biotechnologies to improve product performance in sustainable agriculture.

Materials and methods

Plant materials

Young leaves of *S. miltiorrhiza.* were used as explants to produce in vitro seedlings through callus induction and subsequent clumped buds. The seedlings were cultured under controlled conditions with a day temperature of 25 °C, a night temperature of 20 °C, and a light intensity of 6000 lx with a 12-h light/dark cycle. To minimize the influence of soil microorganisms and to facilitate nutrient control, bayite rock from Ximeishan Village, Zhongjiang County, Deyang City, Sichuan Province (E: 104.54°, N: 30.94°) was selected as the culture medium. The rock, with a particle size of 1–5 mm, was sterilized by dry heat at 180 °C for 4 h and used at a weight of 200 g per culture flask, supplemented with 50 ml of ½ MS medium.

Fungal materials

The five strains in the experiment were isolated from *S. miltiorrhiza*. It has been demonstrated that these strains are non-lethal to test-tube seedlings and have been identified as belonging to four distinct species. (Fig. 1).

Experiment 1: greenhouse experiment

The spore suspension with the same concentration (10^7 CFU·mL^{-1}, 5 mL/plant) was administered by the soil impregnation method, and the inoculation was repeated three times every 2 days. After inoculating spore suspension for 7 days, the seedlings roots of *S. miltiorrhiza* were collected, the DNA was extracted, and then ITS sequencing was performed (Tingke, Sichuan), the PCR amplification primers were ITS1 and ITS4 universal primers. It was confirmed that endophytic fungi had been successfully colonized (TBtools-Blast Zone, v1.09876).

After 30 days of co-culture, aboveground (AW) and underground (UW) fresh weights were measured. The activities of SPS (sucrose phosphate synthase), CAT (catalase), SS (sucrose synthase), GOGAT (glutamate synthase), SOD (superoxide dismutase), GS (glutamine synthetase), PAL (phenylalanine ammonialyase), NR (nitrate reductase) and the contents of Ss (soluble sugar),

St (starch), SP (soluble protein), and MDA (malondialdehyde) in leaves were determined. All plant tissue enzyme activity or content were in accordance with content determination kit instructions steps (Keming, Suzhou). One seedling per bottle and ten replicates per treatment group.

Experiment 2: field experiment

Harvesting, plant biomass, and photosynthetic parameter confirmed the colonization and transplanted to Ximeishan Village (E: 104.54°, N: 30.94°). Each treatment group was treated ten times and harvested 27 weeks later. The fresh weight of the AW (aboveground), UW (root), and DW (root dry weight) was determined. Underground parts were dry-blanching at 105 °C for 30 min, dry at 60 °C to constant weight and measured the dry weight of the sample (DW), and stored for HPLC analysis. The Pn (net photosynthetic rate) of fully spread mature leaves was measured by Li6400 photosynthetic apparatus (Li-COR, Lincoln, NE, USA) from 10:00 to 12:00 on a sunny morning in June and November, the location was in the middle of the leaf and away from the main vein.

Enzyme activities The activities of SS, SPS, SOD, peroxidase (POD), CAT, PAL, GOGAT, GS, NR, nitrite reductase (NiR), glucose dehydrogenase (GDH), glutaminase (GLS), and the contents of Ss, St, SP, MDA, and proline (PRO) in leaves were determined in June, July, August, September, and November. All plant tissue enzyme activity or content was in accordance with content determination kit instructions steps (Keming, Suzhou).

Bioactive compounds The active component contents of dry root were determined by UPLC (due to the limited sample size, metabolites were measured only for field experiment and not for greenhouse experiment), including CA (caffeic acid), RA, PCA (protocatechuic aldehyde), MT, CT (cryptotanshinone), DT, DSS (danshensu), SAA, T-I (tanshinone I), T- IIB (tanshinone IIB), SAB, T-IIA. The standards for SAB, CA, CT, DSS, DT, MT, PCA, RA, SAA, T- I, T- IIB and T- IIA were purchased from the Chengdu Alfa Biotechnology Co.,Ltd (Chengdu, China). The standards and samples were prepared according to the guidelines outlined in the Chinese Pharmacopoeia (Pharmacopoeia Committee of P. R. China, 2015). UPLC analysis was conducted using a Thermo Scientific™ UltiMate™ 3000 system (Thermo Fisher Scientific, USA). The mobile phase consisted of 0.02% aqueous phosphoric acid (solvent A) and acetonitrile (solvent B), following this gradient program: 0–2 min, 5%-10% B; 2–6.5 min, 10%-22% B; 6.5–10 min, 22%-28% B; 10–12 min, 28% B; 12–14 min, 28%-65% B; 14–19 min, 65% B; 19–21 min, 65%-98% B; 21–25 min, 98% B. The flow rate was 0.4 mL/min, detection wavelength was 280 nm, and sample injection volume was 1 μL.

Un-targeted metabolomics Weighed 0.5 g samples from each group containing 10 mL of solution to identify differential metabolites (DAMs) between the blank and treatment groups. All samples underwent analysis using UHPLC-Q-Orbitrap HRMS (Thermo Fisher Scientific, USA). Subsequent to UHPLC-Q-Orbitrap HRMS detection, relative substance contents were determined. Spectra of secondary fragments were matched against the mzCloud network database and the local traditional Chinese medicine ingredient database OTCML for compound identification. Associations were identified using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Data processing and statistical analysis were conducted using MetaboAnalyst. OPLS-DA was performed in MetaboAnalyst. DAMs were selected based on a p value < 0.05 among metabolites with a VIP (variable importance in projection) > 1.

The co-occurrence network analysis To evaluate the interactions between metabolites and fungi of *S. miltiorrhiza.*, network construction was performed after Spearman correlation analysis of fungal OTUs and DAMs. Spearman correlation coefficients r > 0.8 (positive correlation) and r < −0.8 (negative correlation) (p value < 0.001) were considered for significant correlations. All relevant OTUs and differentiated metabolites were visualized in a network, where the correlations were set as edges, OTUs and metabolites were set as nodes. The degree of a node is denoted by its size. Calculated the Spearman's correlations with R package "corrplot" and visualized with Gephi (v 0.9.2) (Bastian et al., 2009).

Statistical analysis

All results are presented as the mean of three replicates ± SE (standard error). Duncan's test and ANOVA (analysis of variance) were used to compare statistical differences between treatment groups. All calculations were done in GraphPad prism 9.0.

Results

Experiment 1: greenhouse experiment

Effects of endophytic fungi inoculation on the biomass of S. miltiorrhiza

Co-cultured with different strains or SynComs in culture flasks for 30 days, the growth and biomass changes of greenhouse seedlings after harvesting are shown in Fig. 2. As a whole, the seedling growth of *S. miltiorrhiza* was in good condition (Fig. 2A). Compared with the CK, SM5 significantly reduced the aboveground part of *S. miltiorrhiza* biomass, SM4 significantly decreased the underground part biomass, and other strains had no significant impact on biomass. SynCom 1 significantly increased the aboveground and underground biomass of *S. miltiorrhiza*

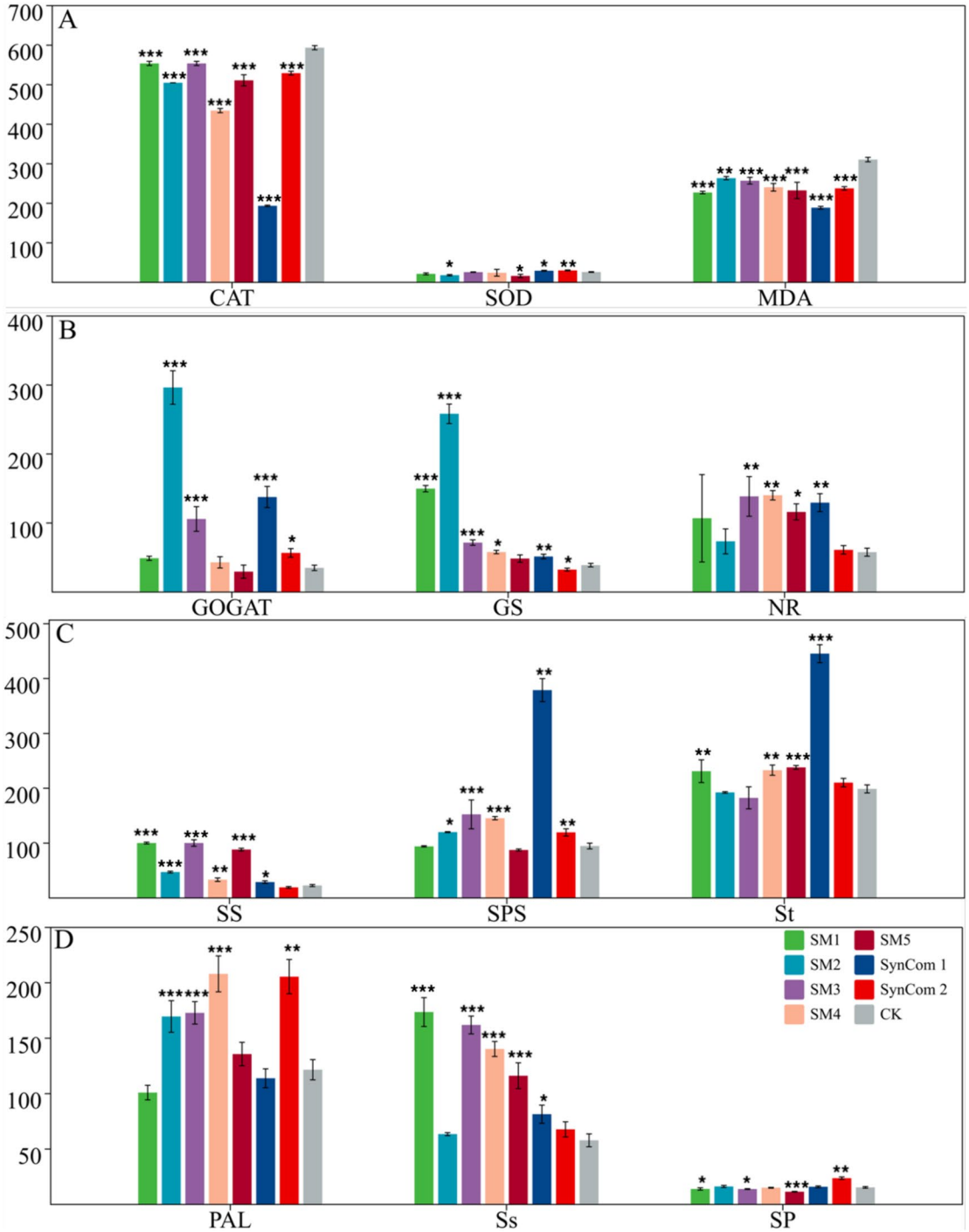


Fig. 3 Effect of endophytic fungi inoculation on enzyme activity of *S. miltiorrhiza*. *CAT* catalase (μmol•min^{-1}•g^{-1}•FW), *SOD* superoxide dismutase (U•g^{-1}•h•FW), *MDA* malondialdehyde (μmol•g^{-1}•FW), *GOGAT* glutamate synthase (U•g^{-1}•FW), *GS* glutamine synthetase (μmol•min^{-1}•g^{-1}•FW), *NR* nitrate reductase (μmol•h^{-1}•g^{-1}•FW), *SS* sucrose synthase (μmol•min^{-1}•g^{-1}•FW), *SPS* sucrose phosphate synthase (μmol•min^{-1}•g^{-1}•FW), *St* starch (μg•g^{-1}•FW), *PAL* phenylalanine ammonialyase (U•mg^{-1}•h), *Ss* soluble sugar (μg•g^{-1}•FW), *SP* soluble protein (μg•ml.$^{-1}$). *p value < 0.05, **p value < 0.01, ***p value < 0.001

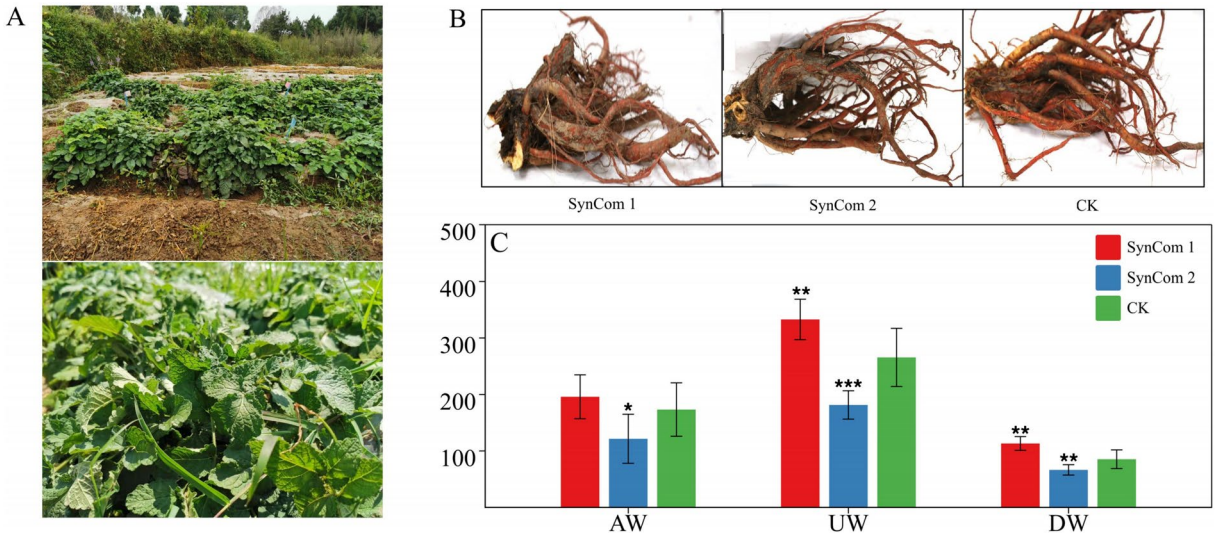


Fig. 4 Effects of endophytic fungi inoculation on the biomass of *S. miltiorrhiza*. **A** The field condition. **B** The growth of filed seedlings. **C** AW, UW and DW biomass (g). **p* value < 0.05, ***p* value < 0.01, ****p* value < 0.001. AW: the fresh weight of the abovegroud, UW: the fresh weight of the root, DW: the dry weight of the root

seedlings by 22% and 56%, respectively. SynCom 2 significantly reduced the aboveground and underground biomass by about 17% and 27%, respectively (Fig. 2B), indicating that SynCom 1 promoted the growth of *S. miltiorrhiza*, while SynCom 2 inhibited the growth.

Effect of endophytic fungi inoculation on enzyme activity of S. miltiorrhiza

To clarify the effects of strains and SynComs on carbon and nitrogen metabolism and resistance of *S. miltiorrhiza*, we determined the activity of related key enzymes and the content of products. Among single strains, SM2 and SM4 significantly promoted most of the enzymes, among which SM2 had the most significant promotion effect on the activities of GOGAT and GS, and SM4 had the most significant impact on NR and PAL activities.

SynCom 1 significantly increased the activity of critical enzymes or the products related to carbon and nitrogen metabolism, among which the activity of SPS was about 4.0 times that of CK, and the accumulation of starch (St) was about 2.2 times that of CK. Interestingly, SynCom 2 had more pronounced effects on resistant enzymes and osmotic substances, where SOD activity increased by about 15% and SP accumulation increased by about 54% (Fig. 3). In conclusion, SynCom 1 may enhance biomass accumulation by promoting the carbon and nitrogen metabolism of *S. miltiorrhiza* seedlings under greenhouse conditions, while SynCom 2 may cause more severe stress to *S. miltiorrhiza* seedlings, thus enabling the plant to increase the activity of defensive enzymes to resist stress.

Experiment 2: field experiment

Effects of SynComs inoculation on the biomass of S. miltiorrhiza

The two SynComs were inoculated and transplanted to Zhongjiang for 27 weeks. After harvesting, the growth and yield changes of the field seedlings are shown in Fig. 4. Compared to the CK, the growth differences under the SynComs treatments were more pronounced, and the underground part was larger under SynCom 1 treatment (Fig. 4B). The results of the field experiment and greenhouse experiment were consistent, SynCom 1 significantly increased the UW and DW of *S. miltiorrhiza* by about 25% and 33%, respectively, SynCom 2 significantly reduced seedling AW, UW, and DW of *S. miltiorrhiza* by 30%, 32%, and 22%, respectively (Fig. 4C). The results showed that SynCom 1 had favorable stability and could promote the growth of *S. miltiorrhiza* in field planting.

Effects of SynComs on photosynthesis and enzyme activities of S. miltiorrhiza.

Compared with June, Pn in November all decreased (Fig. 5A). Similar to the greenhouse experiment, SynCom 1 significantly promoted the related indexes of carbon and nitrogen metabolism; in addition, SynCom 1 antagonistic index showed better positive effect than SynCom 2 in field cultivation (Fig. 5B). PAL is a crucial enzyme in the salvianolic acid secondary metabolic pathway, SynCom 2 has the highest PAL enzyme activity for five consecutive months (Fig. 5m), suggesting that SynCom 2 may promote the synthesis of salvianolic acid. These results suggest that similar to the results of greenhouse

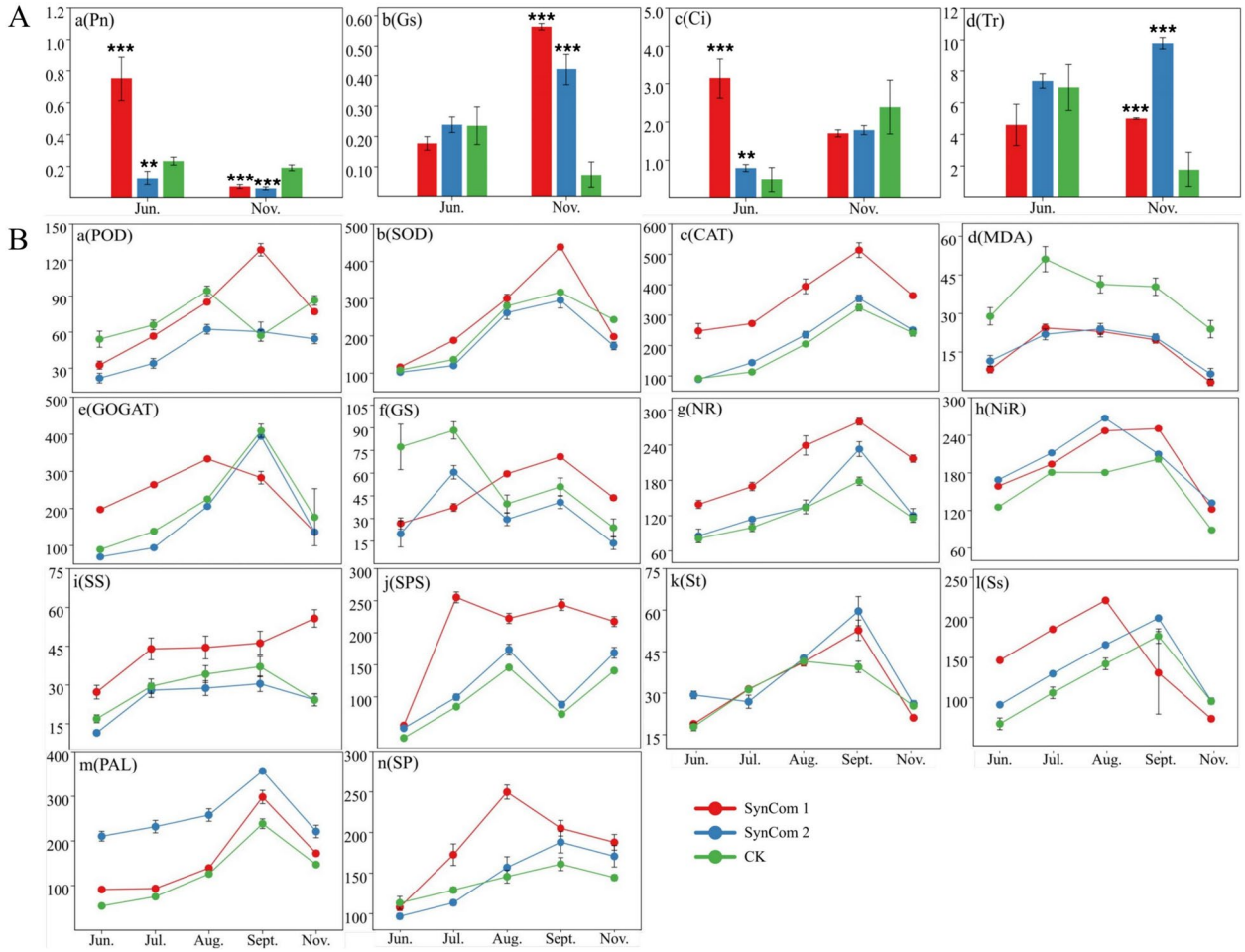


Fig. 5 Effect of SynComs inoculation on enzyme activity of *S. miltiorrhiza*. **A** Pn: net photosynthetic rate (μmol•mss^{-1}), *Gs* stomatal conductance (mmol•ms^{-1}), *Ci* intercellular CO_2 concentration (μmol•mol^{-1}), *Tr* transpiration rate (mmol•ms^{-1}). **B** *POD* peroxidase (U•g^{-1}•h•FW), *SOD* superoxide dismutase (U•g^{-1}•h•FW), *CAT* catalase (μmol•min^{-1}•g^{-1}•FW), *MDA* malondialdehyde (μmol•g^{-1}•FW), *GOGAT* glutamate synthase (U•g^{-1}•FW), *GS* glutamine synthetase (μmol•min^{-1}•g^{-1}•FW), *NR* nitrate reductase (μmol•h^{-1}•g^{-1}•FW), *NiR* nitrite reductase (μmol•min^{-1}•g^{-1}•FW), *SS* sucrose synthase (μmol•min^{-1}•g^{-1}•FW), *SPS* sucrose phosphate synthase (μmol•min^{-1}•g^{-1}•FW), *Ss* soluble sugar (μg•g^{-1}•FW), *St* starch (μg•g^{-1}•FW), *PAL* phenylalanine ammonialyase (U•mg^{-1}•h), *SP* soluble protein (μg•ml^{-1}).*p value < 0.05, **p value < 0.01, ***p value < 0.001, *ns* no significance

experiments, increasing photosynthetic rate and fostering nitrogen and carbon metabolism are also the mechanisms of SynCom 1 promoting the development of *S. miltiorrhiza* in the field.

Effect of SynComs on active components of S. miltiorrhiza

The iconic components for evaluating the quality of *S. miltiorrhiza* are mainly salvianolic acid and tanshinone; thus, enhancing the accumulation of these two main components has become one of the goals of *S. miltiorrhiza* breeding.

SAB content was the highest, PCA content was the lowest, and SynCom generally increased the content of active ingredients in *S. miltiorrhiza*. Except DT, SynCom 1 had significant effects on the other 11 active ingredients, in which the contents of PCA, CA, SAA, SAB, and RA were significantly reduced, and the contents of CT, T-IIA, T-I, MT and T-IIB were about 1.5, 1.3, 1.6, 1.4, and 1.3 times of those in the CK, respectively. Except for DSS, SynCom 2 had significant effects on the other 11 active ingredients. Only PCA and CA contents ere remarkable reduced, and the content of other components was notably increased, the content of SAA and SAB was about 2.7 and 1.9 times that of CK, respectively. DT, CT, T-I, T-IIA, MT, and T-IIB contents were 1.3, 1.6, 1.9, 1.6, 1.5, and 1.6 times those in CK, respectively (Fig. 6). In general, the endophytic fungi SynComs exerts a stronger promotional effect on the synthesis and accumulation of tanshinones than phenolic acids, with SynCom2 being the

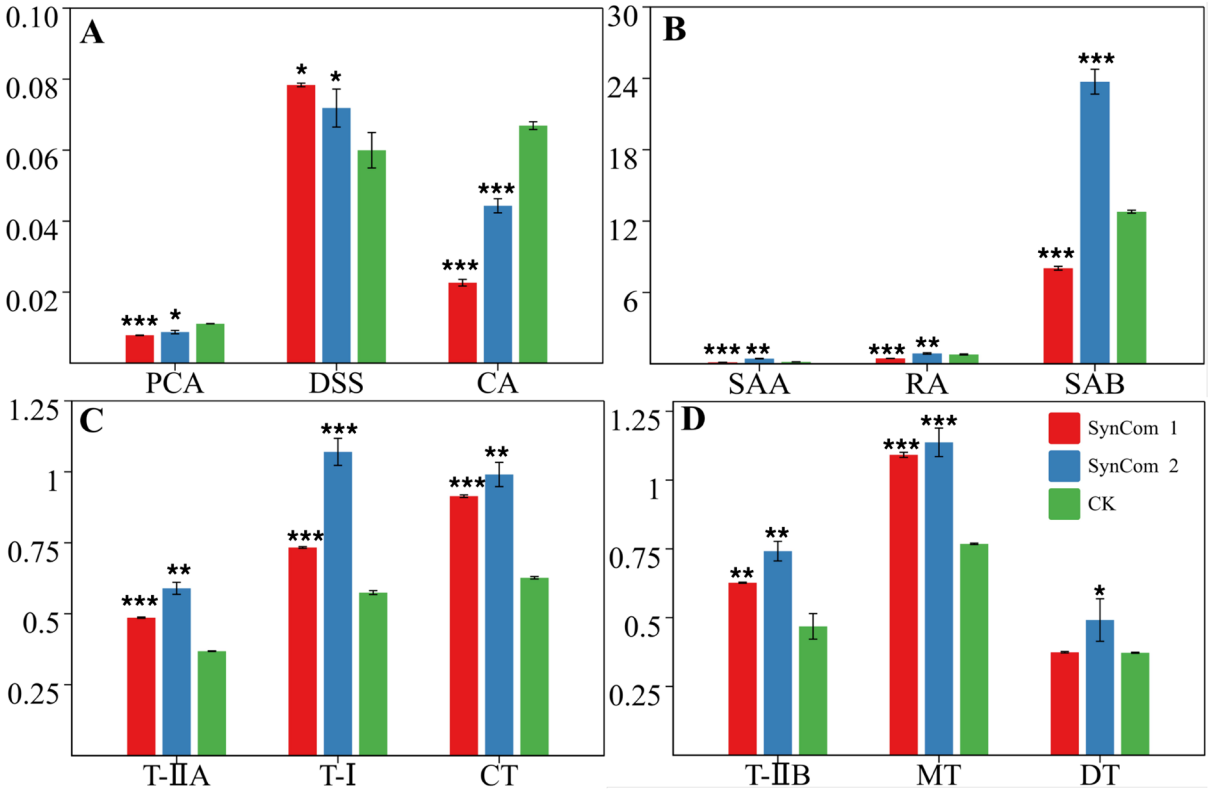


Fig. 6 Effect of SynComs on active components of *S. miltiorrhiza* (mg•g^{-1}). *PCA* protocatechuic aldehyde, *DSS* danshensu, *CA* caffeic acid, *SAA* salvianolic acid A, *RA* rosmarinic acid, *SAB* salvianolic acid B, *T- IIA* tanshinone IIA, *T- I* tanshinone I, *CT* cryptotanshinone, *T- IIB* tanshinone IIB, *MT* miltrione, *DT* dihydrotanshinone. **p* value < 0.05, ***p* value < 0.01, ****p* value < 0.001, ns: no significance

dominant community in enhancing the content of active components.

The correlations between metabolites and SynComs

To illustrate the relationship of biomass, active components, and metabolism indexes, the correlation analysis among all indexes of *S. miltiorrhiza* in the field was carried out, and it was found that the enzymes related to carbon and nitrogen metabolism with the accumulation of biomass and yield of *S. miltiorrhiza* show positive relationship significantly, and significantly negative correlation with the production of secondary metabolites. The content of secondary metabolites was mainly negatively correlated with stress-resistant enzymes such as SOD, and positively correlated with nitrogen metabolism and PAL enzyme activity (Fig. 7-A).

To analyze the differences of metabolites and pathways in *S. miltiorrhiza* which inoculated SynComs or not, LC–MS/MS was used to conduct non-targeted metabolomic analysis of the chemical components of SynCom 1, SynCom 2, and CK. VIP in OPLS-DA was used to find the differential metabolites of biological significance (VIP > 1). The significance of differences between SynComs and CK ($p < 0.05$) was screened with *T* test. A total of 68 metabolic pathways were annotated by KEGG pathway enrichment analysis, of which 13 were significantly enriched ($p < 0.05$). The number of pathways annotated by SynCom 1, SynCom 2, CK were 8, 11, 8, respectively. There are five extremely significant pathways, among which the most significantly enriched pathways of SynCom 1 and SynCom 2 are alanine, aspartic acid, and glutamate, while the most significantly enriched pathway of CK is butyrate (Fig. 7B). The differences in metabolites (DAMs) between SynCom 1 VS CK, SynCom 2 VS CK, and SynCom 1 VS SynCom 2 are 176, 182, 189, which indicated that the metabolic profiles between SynComs and CK were significantly different (Fig. 7C).

To further explore the interaction between SynComs and annotated DAMs, network analysis was performed using fungal OTUs based on positive and negative Spearman's correlations (Fig. 8). SynCom 1 comprised 48 nodes and 132 edges showing significant (p value < 0.001) and strong correlations (r > 0.8

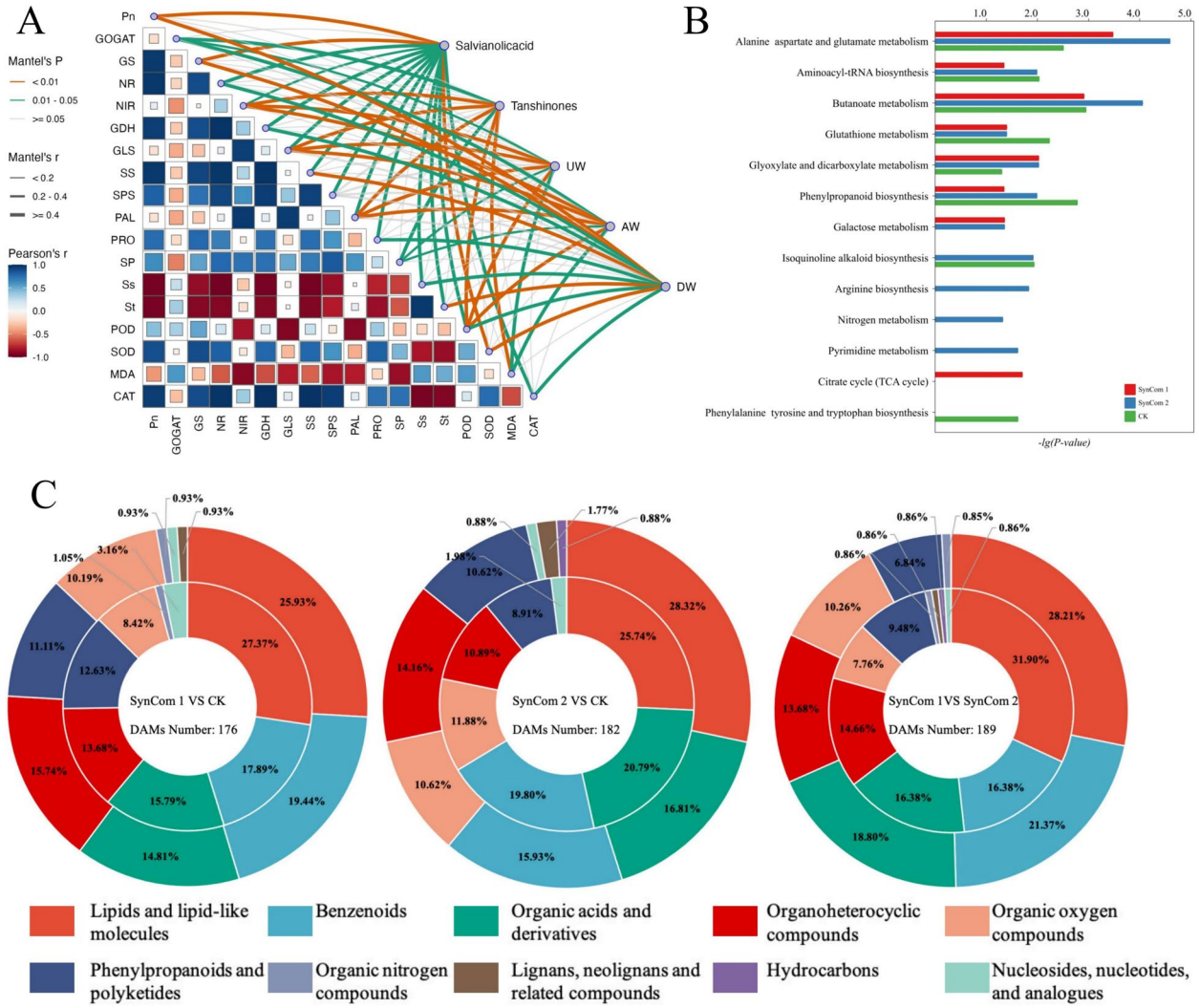


Fig. 7 Metabolome differences among SynComs and correlation analysis between physiological indicators of *S. miltiorrhiza*. **A** Correlation among yield, active components, and enzyme activities of *S. miltiorrhiza*. **B** Differences in SynComs metabolism. **C** Classification of differential metabolites in pairwise comparisons with different treatments. Indicators with insignificant correlations are not shown. DAMs: differential metabolites

or r < -0.8), while SynCom 2 included 75 nodes and 203 edges. In SynCom 1, SM2 and SM4 emerged as key nodes closely connected with other DAMs. The presence of SM3 enhanced the connectivity of SM1, SM4, and SM5 in the network. Furthermore, we analyzed DAMs that strongly interact with SynComs, annotating a total of 57 pathways in KEGG. SynCom 1 unique metabolic pathways were predominantly associated with plant growth (map00020, map01200, map00720, map01070), whereas SynCom 2 was primarily annotated to pathways related to plant secondary metabolism (map00770, map00240, map00290, map00905).

In addition, compared to CK, treatment with SynComs altered the primary and secondary metabolic activities of *S. miltiorrhiza.* SynCom 1 primarily enhanced carbon and nitrogen metabolism, as well as photosynthesis in *S. miltiorrhiza*, thereby promoting plant growth. In contrast, SynCom 2 predominantly influenced the phenylpropanoid and tanshinone metabolic pathways, resulting in increased levels of the major active components (Fig. 9).

Discussion

Assessing crop yield and quality in the field is crucial for evaluating the efficacy of SynComs [37]. Many previous studies have limited their experiments

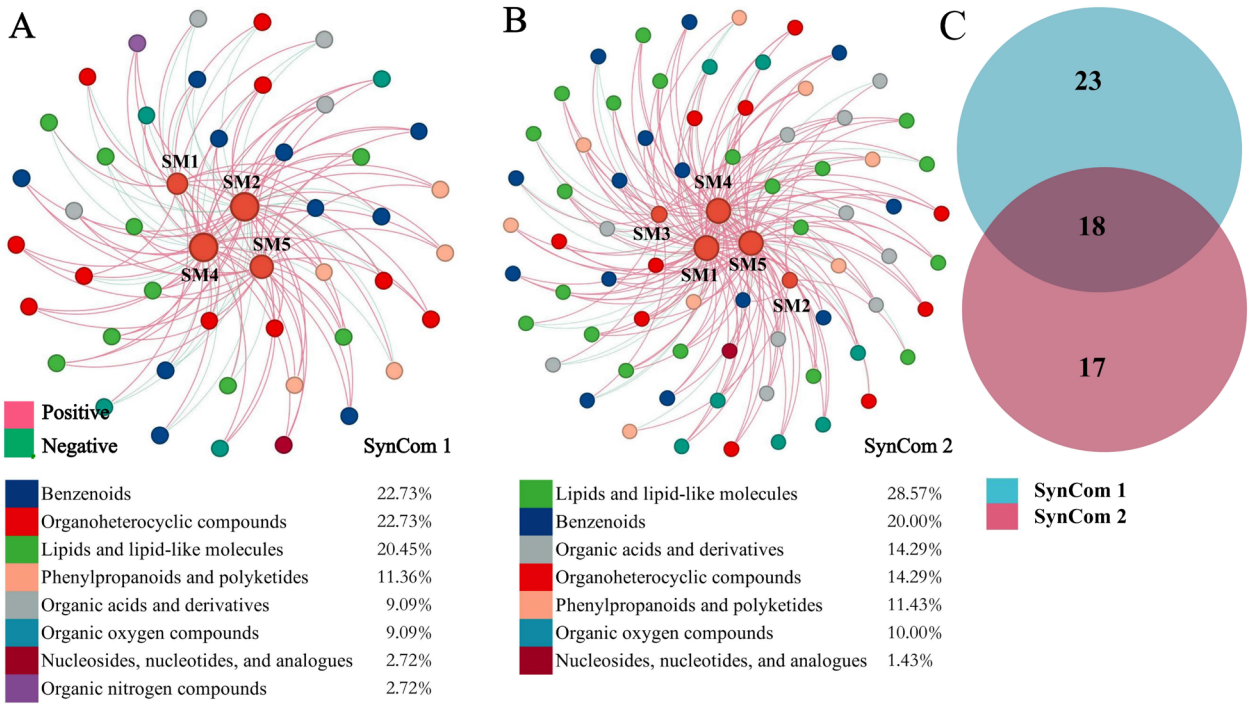


Fig. 8 The correlations of metabolites and microbe using the Spearman's method ($p < 0.001$, $r < -0.8$ or > 0.8). Each node corresponds to a metabolite or an OTU, and edges between nodes correspond to either positive (red) or negative (green) correlations. Network showing the correlation of metabolites and fungal OTUs. **A** SynCom 1, **B** SynCom 2, **C** Comparative analysis of the pathways of DAMs at each treatment. Venn diagram portrays the differences and similarities between each comparison group. Arabic numerals represent the quantity of metabolites. DAMs: differential metabolites

to laboratory or pot/glasshouse trials, overlooking uncontrollable factors arising from natural conditions. This limitation hinders the practical application of SynComs in field settings [6]. None of the five endophytic fungi used in this study individually exhibited significant effects on plant biomass under controlled conditions. However, two synthetic communities (SynComs) demonstrated notable impacts on biomass. To validate the efficacy of our designed SynComs in field crop production, we conducted field experiments. The results consistently demonstrated the stability of SynCom function, with SynCom 1 and SynCom 2 significantly enhancing the yield and quality of *S. miltiorrhiza*, respectively. These findings underscore the potential of the SynComs employed in this study as effective strategies for promoting growth and enhancing quality. Furthermore, the distinct functional differences observed between single bacteria and SynComs suggest that the mechanisms of action of non-growth-promoting microorganisms differ from those of individual bacteria, indicating it is not a simple functional superposition. This highlights that the strain promotion effect on plant growth is not the sole selection criterion in the assembly of SynComs. [12, 13].

Un-targeted metabolic verified that SynCom 1 and SynCom 2 focused on primary and secondary metabolism, respectively, to regulate the yield and quality of *S. miltiorrhiza*. A great quantity of studies have confirmed that the intensity of photosynthesis and carbon and nitrogen metabolism is the key to plant biomass accumulation [14, 16, 30]. In the field experiment, the nutrient competition between plant and microbial community was intensified. Under SynCom 1 treatment, the content of metabolites related to TCA cycle increased and carbon metabolism was enhanced, indicating that SynCom 1 enhanced energy accumulation by promoting the TCA cycle so as to promote plant biomass to compete with fungi for resources [5]. Nitrogen has a significant impact on plant photosynthesis and the distribution of carbon-assimilating substances in herbal medicine [19, 21]. Correlation analysis results showed that the activity of nitrogen metabolizing enzymes was positively correlated with net photosynthetic rate (Fig. 7A), that is, the higher the enzyme activity, the stronger the net photosynthetic rate, which is more conducive to the growth and development of *S. miltiorrhiza*. Un-targeted metabolomics showed

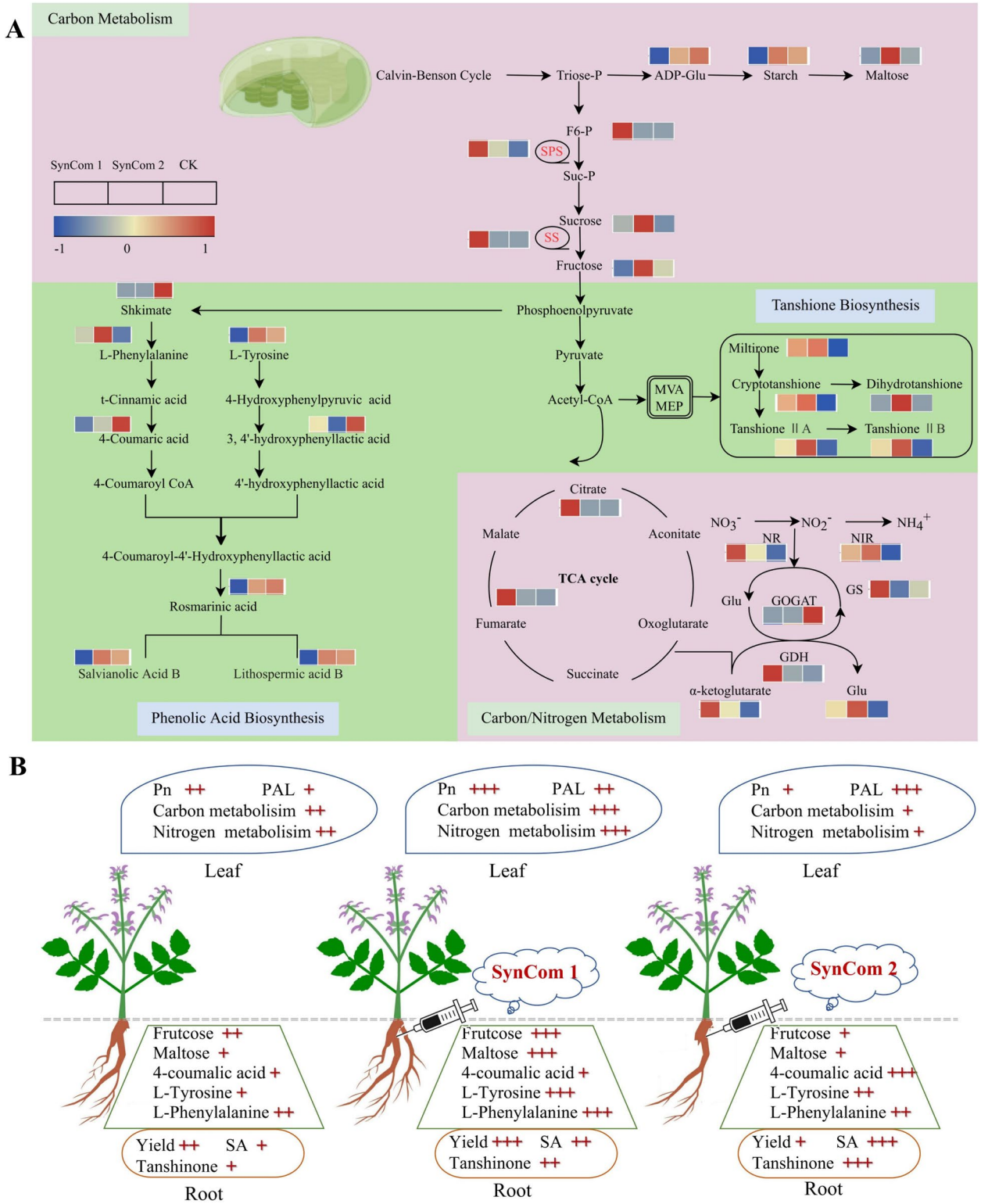


Fig. 9 Effects of SynComs on the metabolism of *S. miltiorrhiza*. **A** Metabolic networks of the DAMs. The metabolic pathways were drawn in accordance with the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, the purple background is primary metabolism and the green background is secondary metabolism. **B** Conceptual model of SynCom structure regulating growth and development of *S. miltiorrhiza*

that SynCom 1 significantly increased the conversion of ammonium nitrogen into plants (Fig. 9). Therefore, SynCom 1 enhanced photosynthetic characteristics by promoting nitrogen utilization, which was beneficial to the growth of *S. miltiorrhiza* [29, 31].

PAL is a crucial enzyme in the synthesis pathway of salvianolic acid. Glucose, the degradation product of sucrose, can produce phenylalanine and tyrosine through the pathway of shikimate. Tyrosine and phenylalanine synthesize the precursor substance RA of salvianolic acid B through tyrosine derivative branch and phenylpropanoid branch [25, 26]. This study confirmed that salvianolic acids were significantly positively correlated with PAL activity, soluble sugar, and starch content (Fig. 7A). SynCom 2 increased the contents of tyrosine and phenylalanine (Fig. 8), indicating that the accumulation of carbohydrates promoted the synthesis of phenylalanine and tyrosine, thereby promoting the synthesis of salvianolic acids. Drawing from these findings, it is concluded that the significant increase in substrate content correlated with the accumulation of metabolites which might account for the SynCom 2 that heightened the yield of active ingredient in *S. miltiorrhiza*.

In a shared habitat, microorganisms engage in competition for space and nutrient resources through nutritional and antagonistic interactions. Interspecific competition serves as a crucial selection process that significantly influences the functioning of microbial communities [2, 32, 33]. The five strains selected for this study belonged to two families, with the four strains comprising SynCom 1 originating from the same family (Fig. 1). Under greenhouse conditions, SynCom 1 significantly increased biomass, suggesting a relationship attributable to the genetic proximity among its constituent four strains. This supports the hypothesis that closely related species share common secreted substances rather than competing for nutrients [23]. Compared to SynCom 1, the differential strain SM3 in SynCom 2 originated from a different family. Our results demonstrated that inoculation with SynCom 2 increased the production of osmotic substances and antioxidant enzymatic activity (Fig. 3), suggesting that *S. miltiorrhiza* was subjected to greater stress. This phenomenon could be attributed to intensified interspecific competition among the five strains comprising SynCom from various families [8, 24]. According to the network analysis, the presence of SM3 promotes the interaction between strains and metabolites, suggesting that the addition of distant species can change the interaction pattern among closely related species. These results suggest that the genetic relationships among strains can be considered as one of the considerations for development of synthetic communities.

Conclusion

In summary, our findings suggest that the promotion of plant growth by strains is not the sole criterion for designing SynComs. Even SynComs composed of fungi that do not significantly enhance growth can potentially stimulate the development of medicinal plants. SynCom 1 enhanced carbon–nitrogen metabolism processes to increase yield, while SynCom 2 enriched substrate contents in salvianolic acid and tanshinone synthesis pathways to improve quality. This study is valuable for further exploring the application potential of microorganisms with less obvious functions. Furthermore, these SynComs exhibit latent capacities for sustainable application in field cultivation.

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Author contributions

Z-YY conceptualized and designed the experiment, and received financial support. H-MJ and Y-RW are responsible for preparing the initial draft of the manuscript. C-WZ and HW have revised the manuscript. All authors conducted experiments. All authors have contributed to the article and approved the submitted version.

Availability of data and materials

No datasets were generated or analyzed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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