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α -Terpineol fumigation alleviates negative plant-soil feedbacks of *Panax notoginseng* via suppressing Ascomycota and enriching antagonistic bacteria

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Abstract

The accumulation of soil-borne pathogens is the main driving factor of negative plant-soil feedbacks (NPSFs), which seriously restricts the sustainable development of agriculture. Using natural volatile organic compounds (VOCs) from plants or microorganisms as biofumigants is an emerging strategy to alleviate NPSFs in an environmentally-friendly way. Here, we identified α -terpineol from the VOCs of pine needles, confirmed the ability of α -terpineol fumigation in alleviating the NPSF of *Panax notoginseng* via significantly reducing seed decay rate, and also deciphered the underlying mechanism by which the soil microbial community is modified. α -Terpineol fumigation could suppress culturable fungi but enrich bacteria in a dose-dependent manner. Network analysis with high-throughput sequencing data revealed that α -terpineol could distinctly modify both fungal and bacterial communities. In detail, α -terpineol significantly suppressed the relative abundance of Ascomycota from 64.04 to 32.26%, but enriched the relative abundance of Proteobacteria, Acidobacteria and Actinobacteria. Subnetwork analysis further demonstrated that α -terpineol could directly or indirectly suppress fungal pathogens and enrich plant growth-promoting rhizobacteria (PGPRs). *In vitro* fumigation and co-culture experiments with culturable isolates validated these findings. The antagonism between beneficial bacteria and pathogens, and the synergistic growth promotion among α -terpineol-enriched bacteria might be involved in soil microbial community assembly. In summary, α -terpineol fumigation could directly or indirectly modify the soil microbial community to alleviate NPSFs, especially by suppressing fungal pathogens and enriching beneficial bacteria. This study suggests that VOCs from natural products are worth developing as biofumigants due to their multiple functions in modifying the soil microbial community.

Keywords: Negative plant-soil feedbacks, α -Terpineol, Soil-borne pathogens, Microbiome, Network analysis

Background

The processes by which plants alter the biotic and abiotic qualities of their living soils and cause positive or negative effects on the survival of themselves or their offspring are known as plant-soil feedbacks (PSFs)

(Kulmatiski et al. 2008; Bennett et al. 2017; Bennett and Klironomos 2019). Among PSFs, negative plant-soil feedbacks (NPSFs) usually play important roles in maintaining species diversity in natural system (Kulmatiski et al. 2008; Mangan et al. 2010; Lankau et al. 2011), whereas severely restrict the sustainable development of agricultural production, especially in monoculture cropping systems (Huang et al. 2013; Wei et al. 2018). Although some studies indicate that nutrient imbalance, the deterioration of soil physico-chemical properties and

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the accumulation of autotoxins in rhizosphere soil lead to NPSFs, further evidence has demonstrated that imbalances in the rhizospheric microbiome, especially the build-up of soil-borne pathogens and the suppression of plant growth-promoting rhizobacteria (PGPRs), are the main reasons for NPSFs (Mangan et al. 2010; Yang et al. 2015; Wei et al. 2018; Yang et al. 2018a; Liu et al. 2019; Luo et al. 2019; Yang et al. 2019).

Methods to eliminate soil-borne pathogens, including steaming, microwave radiation, gamma irradiation, soil fumigants and fungicides, have been reported as effective ways to alleviate NPSFs (Alphei and Scheu 1993; Katan 2000; Miguel et al. 2004; Klose et al. 2006; Fennimore et al. 2014; Li et al. 2019; Yang et al. 2019). Among these methods, soil chemical fumigation is one of the most effective ways to control soil-borne diseases, which is attributable to the strong ability of the gases used to diffuse through the continuous soil air space, however, environmental unfriendliness has become the major limitation of this method (Lembright 1990; Chen et al. 2018). Although soil-borne pathogens are effectively killed, beneficial soil microorganisms are also suppressed due to the broad-spectrum antimicrobial activities of soil chemical fumigants (Podio et al. 2008; Wang et al. 2014; Gurtler 2017; Li et al. 2019; Yang et al. 2019). Moreover, soil-borne pathogens can quickly recolonize sterile soils in the absence of antagonism from beneficial microbes (Mendes et al. 2011; Yang et al. 2019). In addition, most soil treatment methods can be administered only before planting, which limits their application (Ibekwe et al. 2001; Gimsing and Kirkegaard 2006). Recently, many environmentally friendly fumigants have been developed and applied in the field (Gurtler 2017). Among them, soil biofumigations, which are based on the release of antipathogen volatiles from fresh plant residues (such as broccoli, cabbage and cauliflower) or microbes (such as *Bacillus amyloliquefaciens* and *Streptomyces alboblavus*), have been widely reported in controlling soil-borne diseases (Nuñez-Zoffio et al. 2012; Yuan et al. 2012; Arnault et al. 2013; Schalchli et al. 2016; Gurtler 2017; Yuan et al. 2017; Yang et al. 2018b; Jin et al. 2019; Zhang et al. 2020).

Sanqi (*Panax notoginseng* (Burk.) F. H. Chen), one of the most important Chinese medicinal plants, encounters NPSF-caused replant failure when it is successively planted in the same location (Yang et al. 2015). The build-up of soil-borne pathogens is the main cause of replant failure (Luo et al. 2019). Currently, a new cultivation model in coniferous forests has been developed to alleviate NPSF (Ye et al. 2019). Allelopathic interactions in forest cultivation mode, especially the volatile organic compounds (VOCs) released from pines and withered pine needles, can reduce disease damage by inducing plant SAR (systemic acquired resistance) or directly suppressing the growth of pathogens (Zheng 2008;

Riedlmeier et al. 2017). The VOCs emitted by pines or pine needles consist mainly of monoterpenes and alcohols, such as α -pinene, β -pinene, germacrene D, myrcene, α -terpineol and terpineol-4 (Ucar and Balaban 2004; Kim and Shin 2005; Park and Lee 2011; Politeo et al. 2011). Among them, α -terpineol was reported to inhibit the growth of fungal pathogens by disrupting fungal cell membrane integrity (Park et al. 2009; Pinto et al. 2014; Zhou et al. 2014). However, whether α -terpineol fumigation can alleviate the NPSFs involved in *P. notoginseng* cultivation and the underlying mechanisms remain unclear.

In this study, we used α -terpineol as a biofumigant to treat soil in which a consecutive cultivation of *P. notoginseng* had been carried out, to (i) evaluate the effect of α -terpineol fumigation on alleviating NPSF; (ii) characterize the impacts of α -terpineol on soil bacterial and fungal community, as well as its dose effect; and (iii) explore the relationship between α -terpineol fumigation, microbial community shifts and NPSF alleviation, and decipher the potential underlying mechanisms. We further validated the correlations of microbes shown in the co-occurrence network by *in vitro* fumigation or co-culture. In brief, we expect to explore the mechanism by which α -terpineol fumigation alleviates NPSFs.

Results

α -Terpineol was detected in the volatile compounds released by pine needles

The VOCs from pine needles of *Pinus yunnanensis* collected from three sites at different altitudes were analyzed by gas chromatography-mass spectrometry (GC-MS). α -Terpineol was detected in all samples, with a MS (mass spectra) similarity higher than 90% (Fig. 1a-c) compared with the data in NIST14 library. The presence of α -terpineol in pine needles was further verified by comparing the RT (retention time) and RI (retention index) with the synthetic standard sample of α -terpineol (Fig. 1d).

Alleviation of NPSFs by α -terpineol fumigation

In pot experiments, the seed decay rate in soil with consecutive plantings of *P. notoginseng* reached 90.37% (Fig. 2). It was significantly decreased when the soil was fumigated with 50 μ L/L of α -terpineol for 4 weeks, with a value of 67.50% (Fig. 2). In contrast, the seed decay rate was significantly decreased to 13.28% after steam sterilization at 121 °C for 15 min (Fig. 2). The results suggest that killing the soil-borne pathogens does alleviate NPSFs.

α -Terpineol fumigation suppresses culturable fungi but enriches bacteria

The number of culturable fungi was significantly decreased when consecutively planted soil was fumigated with an increasing concentration of α -terpineol for 4

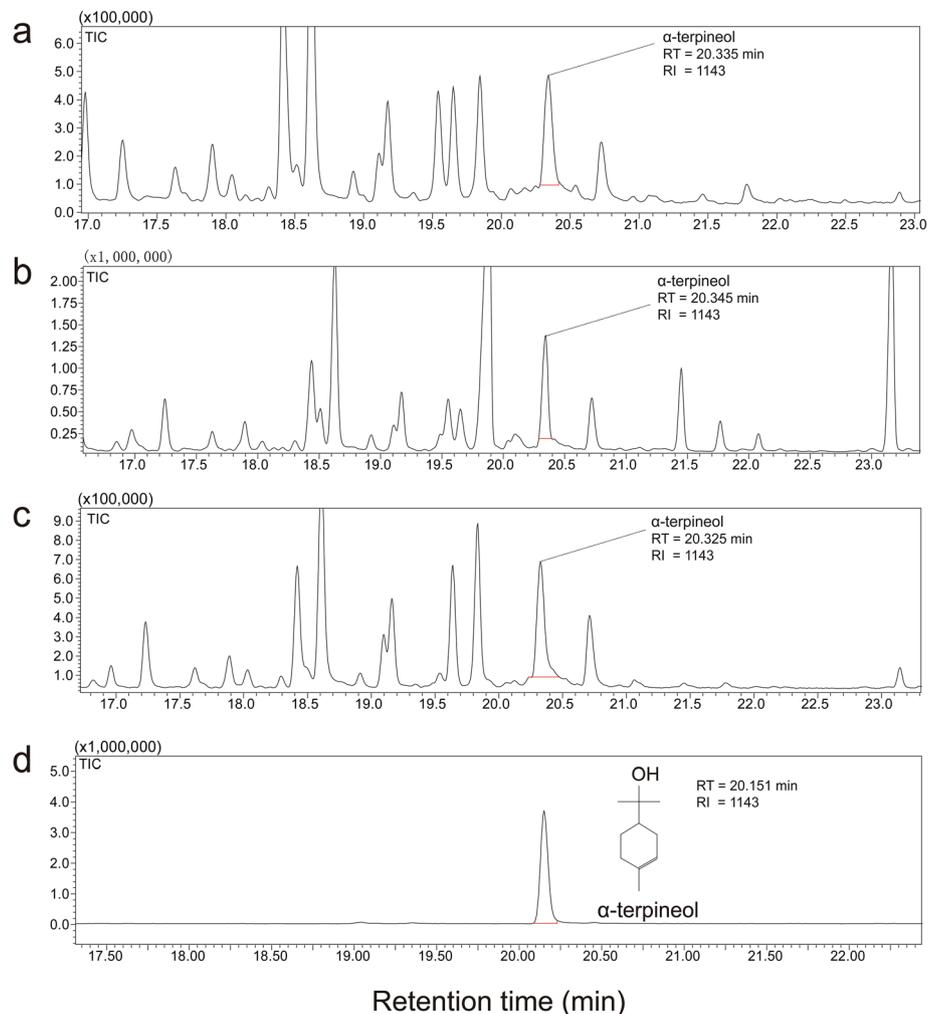


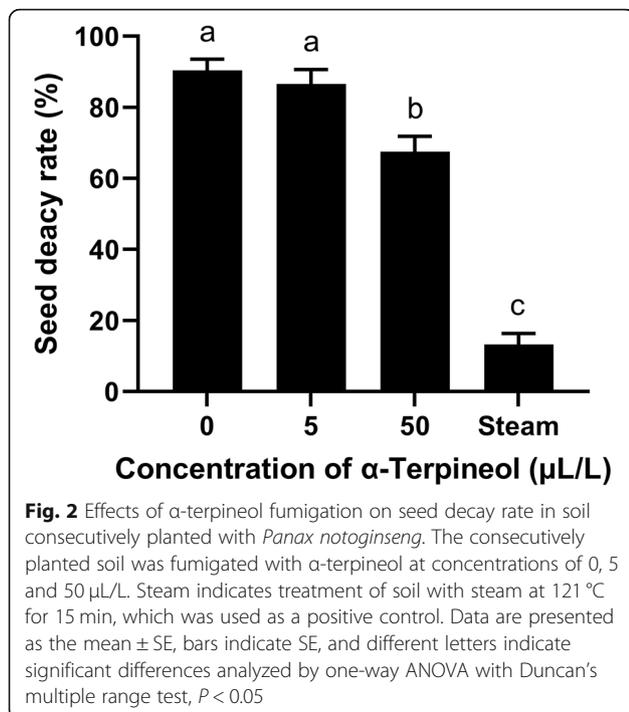
Fig. 1 Total ion current (TIC) chromatogram of VOCs emitted by pine needle powder of *Pinus yunnanensis*. **a** TIC chromatogram of the sample collected from Deqing County (28°2'5"N, 99°29'22"E, 2860 m). **b** TIC chromatogram of the sample collected from Lijiang City (26°57'16"N, 100°3'34"E, 1840 m). **c** TIC chromatogram of the sample collected from Chuxiong City (24°42'37"N, 101°21'17"E, 1640 m). **d** TIC chromatogram from the synthetic standard sample of α -terpineol. RT and RI indicate retention time and retention index, respectively

weeks (Fig. 3a), and the decrease in fungal number was dose-dependent (Pearson's $R = -0.457$, $P < 0.01$). However, the number of cultivable bacteria was significantly increased when fumigated with α -terpineol at concentrations of 5, 20 and 50 $\mu\text{L/L}$ (Fig. 3b). Thus, the ratio of fungi to bacteria gradually decreased with increasing concentrations of α -terpineol (Fig. 3c).

α -Terpineol fumigation changes the diversity and composition of soil microbiome

The consecutively planted soils fumigated with α -terpineol at concentrations of 0, 5, 20 and 50 $\mu\text{L/L}$ were further analyzed with high-throughput sequencing technique to study the changes in fungal and bacterial communities. After filtering, a total of 1,408,373 high-quality reads of fungi were obtained from 12 samples, varying

from 104,826 to 127,157 in each sample, and ultimately yielded 7029 OTUs (operational taxonomic units) with a cut-off level of 97% similarity (Additional file 1: Table S1). For bacteria, we observed that the high abundance of *Escherichia-Shigella* (from 81.96 to 93.02% at the genus level) overshadowed the remaining microbial community (Additional file 2: Figure S1). Because of the weak correlation between *Escherichia-Shigella* and α -terpineol (Pearson's $R = 0.114$, $P = 0.724$), removing the sequence of *Escherichia-Shigella* did not influence the evaluation of α -terpineol on microbial communities (Additional file 2: Figure S2). Accordingly, we eliminated *Escherichia-Shigella* sequences to gain more detailed insights into other microbial associates. Our *Escherichia-Shigella*-free dataset contained a total of 431,237 high-quality reads and yielded 3277 OTUs with a cut-off level

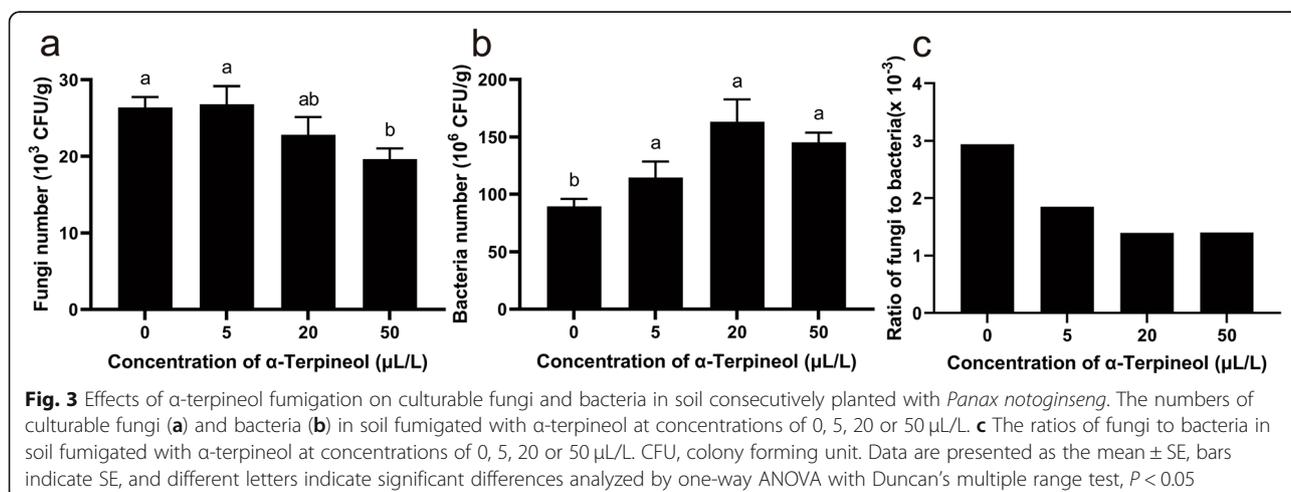


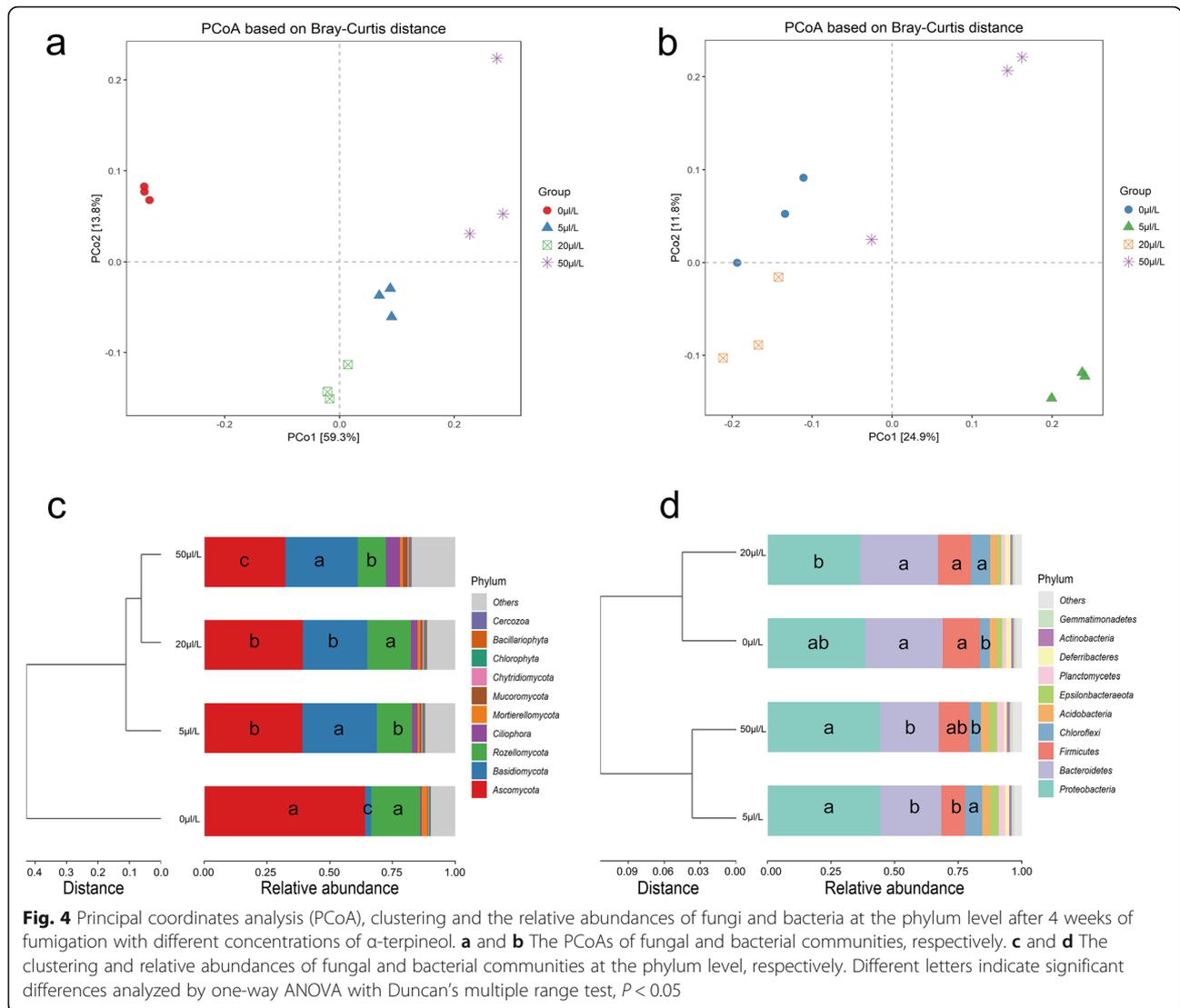
of 97% similarity for further analysis (Additional file 1: Table S2).

Regarding the fungal community, the richness index (Chao1) first increased when soil fumigated with 5 $\mu\text{L/L}$ α -terpineol and then decreased when the concentration of α -terpineol was increased to 20 or 50 $\mu\text{L/L}$. However, the diversity index (Simpson) showed a significant dose-dependent positive correlation with α -terpineol (Pearson's $R = 0.697$, $P < 0.05$) (Additional file 2: Figure S3). Regarding the bacterial community, there were no significant changes in the Chao1 and Simpson indices with increasing concentrations of α -terpineol (Additional file 2: Figure S3).

Principal coordinate analysis (PCoA) based on Bray-Curtis distance indicated that both fungal (Fig. 4a) and bacterial communities (Fig. 4b) were clearly altered with increasing concentrations of α -terpineol. The first axis explained more of the variation in fungal community than in bacterial community (Fig. 4a, b), which indicated the stronger effects of α -terpineol fumigation on fungi. We further found that the fungal community treated by 0 $\mu\text{L/L}$ of α -terpineol was distinctly separated from that by 5, 20 or 50 $\mu\text{L/L}$ of α -terpineol on the first axis, and also that by 5 or 20 $\mu\text{L/L}$ of α -terpineol on the second axis (Fig. 4a). The bacterial community treated by 0 $\mu\text{L/L}$ of α -terpineol was separated from that by 5 or 50 $\mu\text{L/L}$ of α -terpineol on the first axis, and by 5 $\mu\text{L/L}$ of α -terpineol on the second axis (Fig. 4b).

At the phylum level, Ascomycota dominated (64.04%) the fungal community in the consecutively planted soil, followed by Rozellomycota (19.49%) (Fig. 4c). When the consecutively planted soil was fumigated with α -terpineol at 5, 20 and 50 $\mu\text{L/L}$, the relative abundance of Ascomycota was significantly decreased to 32.26–39.34% (Fig. 4c). The same trend was observed in Rozellomycota (11.20–17.33%) (Fig. 4c). The decrease in Ascomycota and Rozellomycota was strongly dependent on the dose of α -terpineol (Pearson's $R = -0.719$, $P < 0.01$ and Pearson's $R = -0.701$, $P < 0.05$, respectively) (Additional file 1: Table S3). In the bacterial community, Proteobacteria (38.46%), Bacteroidetes (30.40%) and Firmicutes (14.59%) occupied the dominant niches in the consecutively planted soil (Fig. 4d). However, there were no significant dose-dependent changes in most phyla upon α -terpineol fumigation. Only weak positive correlations were observed in Proteobacteria, Acidobacteria, Epsilonbacteraeota, Planctomycetes and Actinobacteria with increasing concentrations of α -terpineol (Additional file 1: Table S3).





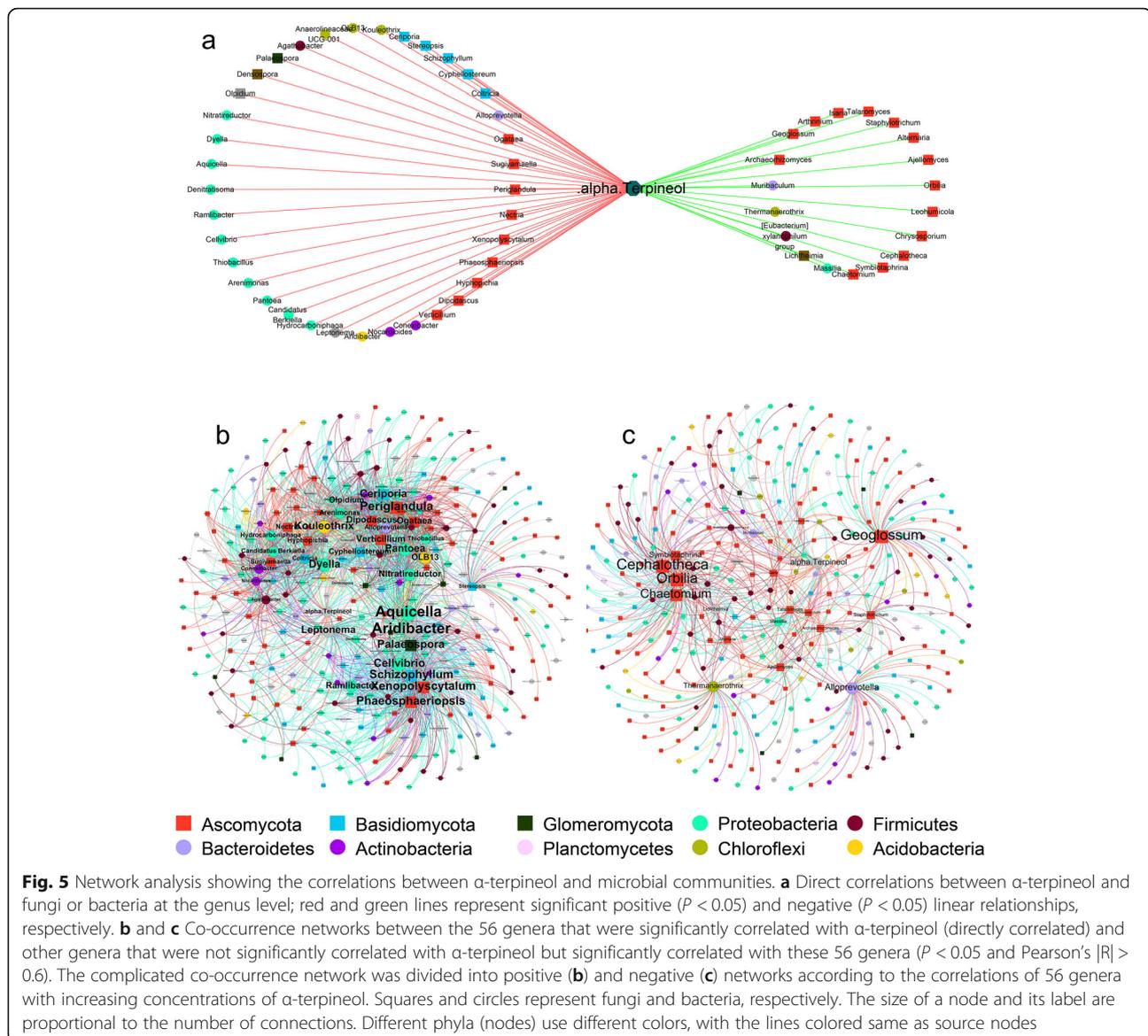
At the genus level, a total of 56 genera from fungi and bacteria were significantly correlated with the increase of α -terpineol ($P < 0.05$), including 32 genera of fungi and 24 genera of bacteria (Fig. 5a). Among them, 37 genera were strongly positively correlated with the increase of α -terpineol, of which 11 genera belonged to Proteobacteria (Fig. 5a). A total of 19 genera were significantly negatively correlated with the increase of α -terpineol, of which 14 belonged to Ascomycota (Fig. 5a). These data further suggest that fungi (especially Ascomycota) are suppressed but bacteria (especially Proteobacteria) are selectively enriched by α -terpineol fumigation.

α -Terpineol fumigation modifies the soil microbial co-occurrence network

The positive and negative co-occurrence networks were almost equal in size (the numbers of nodes were 335

and 343, respectively) (Table 1). However, the positive network was more complex than the negative network (Fig. 5b, c), which reflected higher topological properties, including the total number of links (the edges between two nodes), average clustering coefficient (the higher connectedness among nodes in a particular region of a network), network density and average degree (the number of edges connected to a node) (Table 1).

In the positive network, genera belonging to Proteobacteria accounted for 26.57%, followed by Ascomycota (24.18%) and Firmicutes (11.04%). However, genera of Ascomycota (32.94%) dominated the negative network (Table 1). A node with a high degree (the number of edges connected to a node) is directly correlated with many nodes and is usually considered the keystone taxon of a complex network. Thus, we calculated the degrees of all nodes and



further considered the top 10 genera with the highest degrees as the keystone taxa in the network (Table 1). Seven of the keystone taxa in the negative network and only two in the positive network belonged to Ascomycota (Table 1). *Aquicella* and *Aridibacter* showed the strongest connectivity in the positive network, with the highest degrees (Fig. 5b and Table 1).

α -Terpineol fumigation directly or indirectly suppresses pathogens but enriches PGPRs

The widely reported PGPRs (Additional file 1: Table S4) and soil-borne pathogens of *P. notoginseng* were highlighted in the α -terpineol correlated subnetwork (Fig. 6). *Alternaria*, a potential soil-borne pathogen of *P. notoginseng*, was significantly negatively correlated with

the concentration of α -terpineol (Fig. 6). The common soil-borne pathogen *Phytophthora* showed a negative correlation with *Alloprevotella*, a bacteria positively correlated with α -terpineol (Fig. 6). Another pathogen, *Gibberella*, showed positive correlations with *Staphylo-trichum* and *Geoglossum*, which were significantly negatively correlated with α -terpineol (Fig. 6). Thus, we inferred that these three potential soil-borne pathogens of *P. notoginseng* may have direct or indirect negative correlations with α -terpineol. In contrast, many PGPRs showed direct or indirect positive correlations with α -terpineol (Fig. 6). Among them, *Pantoea*, *Thiobacillus*, *Nocardioidea* and *Cellvibrio* were directly positively correlated with α -terpineol. Other PGPRs, including *Azospirillum*, *Bacillus*, *Enterobacter*, *Aeromonas*, *Klebsiella*, *Stenotrophomonas*, *Frankia*, *Streptomyces*, *Enterococcus*,

Table 1 Topological properties in positive and negative co-occurrence networks

Network topology parameters	Positive (including indirect positive)		Negative (including indirect negative)	
Total nodes	335		343	
Total links	2341		753	
Average clustering coefficient	0.521		0.321	
Network density	0.042		0.013	
Average degree	13.976		4.391	
Percentage of top 10 phyla	Fungi	Bacteria	Fungi	Bacteria
	Ascomycota (24.18%)	Proteobacteria (26.57%)	Ascomycota (32.94%)	Proteobacteria (21.28%)
	Basidiomycota (10.75%)	Firmicutes (11.04%)	Basidiomycota (9.91%)	Firmicutes (12.54%)
	Glomeromycota (2.39%)	Bacteroidetes (5.67%)	Glomeromycota (1.46%)	Bacteroidetes (5.25%)
		Actinobacteria (3.88%)		Actinobacteria (4.08%)
		Planctomycetes (3.58%)		Acidobacteria (2.62%)
		Chloroflexi (2.09%)		Chloroflexi (2.33%)
		Acidobacteria (1.79%)		Plantctomycetes (1.46%)
Keystone taxa (the numbers of degrees) [phylum which the genus belong to]	Fungi	Bacteria	Fungi	Bacteria
	<i>Periglandula</i> (92) [Ascomycota]	<i>Aquicella</i> (105) [Proteobacteria]	<i>Cephalotheca</i> (71) [Ascomycota]	<i>Alloprevotella</i> (50) [Bacteroidetes]
	<i>Phaeosphaeriopsis</i> (90) [Ascomycota]	<i>Aridibacter</i> (105) [Acidobacteria]	<i>Geoglossum</i> (70) [Ascomycota]	<i>Thermanaerthrix</i> (44) [Chloroflexi]
	<i>Ceriporia</i> (84) [Basidiomycota]	<i>Cellvibrio</i> (87) [Proteobacteria]	<i>Orbilia</i> (69) [Ascomycota]	
	<i>Schizophyllum</i> (90) [Basidiomycota]	<i>Dyella</i> (84) [Proteobacteria]	<i>Chaetomium</i> (63) [Ascomycota]	
	<i>Palaeospora</i> (87) [Glomeromycota]	<i>Kouleothrix</i> (84) [Chloroflexi]	<i>Symbiotaphrina</i> (44) [Ascomycota]	
			<i>Staphylotrichum</i> (31) [Ascomycota]	
			<i>Lichtheimia</i> (32) [Mucoromycota]	
			<i>Ajellomyces</i> (31) [Ascomycota]	

Acinetobacter, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* and *Burkholderia-Caballeronia-Paraburkholderia*, showed indirect positive correlations with α -terpineol (Fig. 6). We further found that all these enriched PGPRs showed a negative correlation with the abovementioned three pathogens, i.e. *Gibberella*, *Phytophthora* and *Alternaria* (Fig. 6). Among them, *Bacillus* and *Ramlibacter* were significantly negatively correlated with *Alternaria* (Fig. 6).

To validate the effects of α -terpineol on microbes, we investigated the antimicrobial activities of α -terpineol against five soil-borne pathogens of *P. notoginseng* by *in vitro* fumigation test. The results showed that the mycelial growth of these five pathogens was inhibited by increasing concentrations of α -terpineol (Fig. 7a). Based

on the effective concentration for 50% inhibition (EC_{50}), we found that *Phytophthora cactorum* was strongly inhibited by α -terpineol with an EC_{50} of 22.50 μ L/L, followed by *Alternaria panax*, *Ilyonectria destructans* and *Fusarium oxysporum* with EC_{50} values of 57.16, 66.63 and 106.59 μ L/L, respectively. *Fusarium solani* was less sensitive to α -terpineol than the other four pathogens but was also inhibited strongly (with an EC_{50} value of 305.55 μ L/L). To validate the effects of α -terpineol on other culturable non-pathogenic fungi, we obtained 23 fungal isolates belonging to 8 genera from consecutively planted soil of *P. notoginseng* (Additional file 1: Table S5). Among them, six non-pathogenic fungal isolates identified as *Chaetomium globosum*, *Talaromyces flavus*, *Fusarium redolens* and *Fusarium graminearum* were all

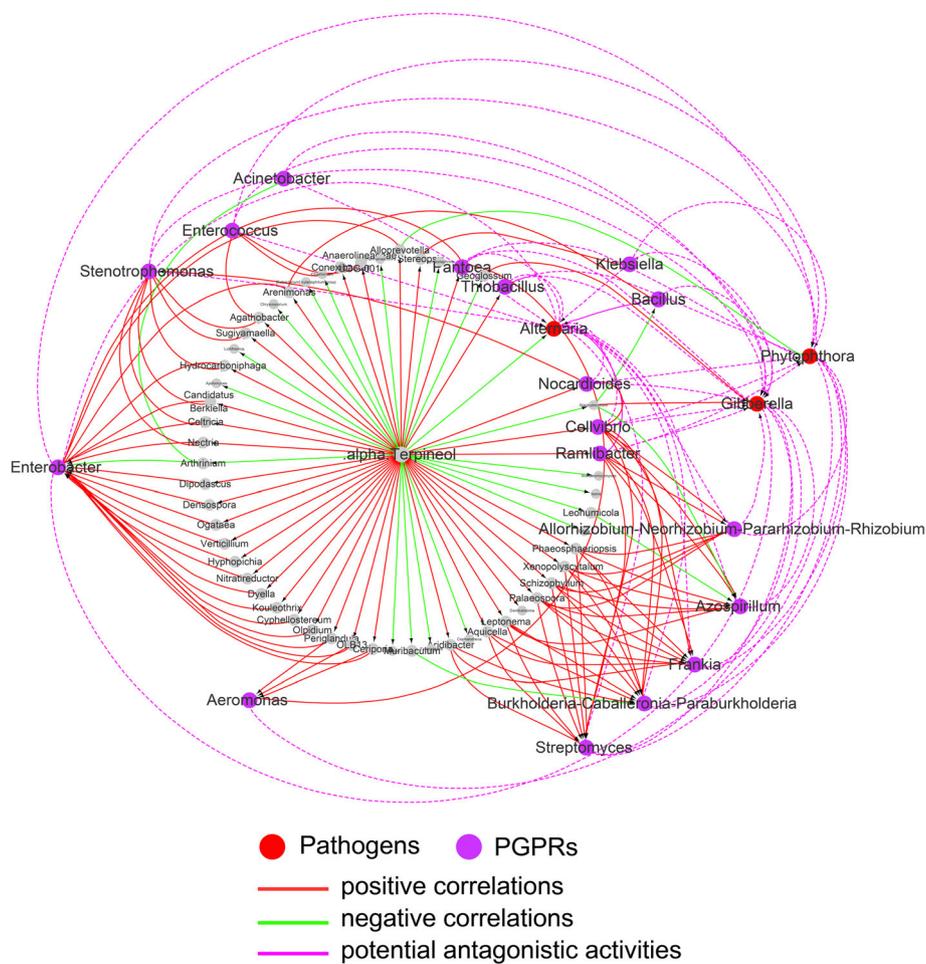


Fig. 6 Subnetwork of 56 genera of fungi and bacteria directly or indirectly correlated with the increase of α -terpeneol, including fungal pathogens and previously isolated or widely reported PGPRs. Purple and red circles represent PGPRs and pathogens, respectively. Red and green lines indicate positive and negative correlations, respectively. The purple line represents the potential antagonistic activities of PGPRs against pathogens. Solid and dotted lines indicate significant and nonsignificant correlations, respectively

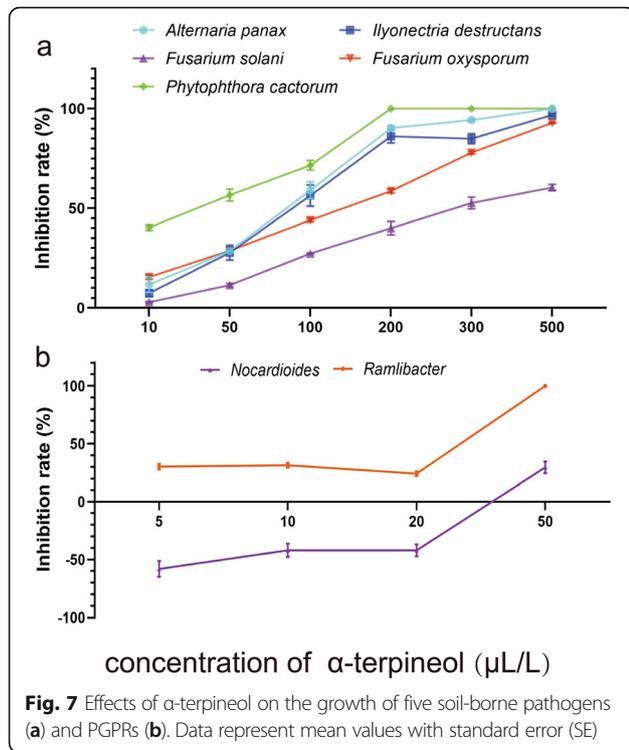
inhibited by increasing concentrations of α -terpeneol with EC_{50} values of 164.31, 63.99, 41.46, 42.40, 79.12 and 31.93 $\mu\text{L/L}$, respectively (Additional file 2: Figure S4).

A total of 189 bacterial isolates belonging to 26 genera (Additional file 1: Table S6) were isolated from consecutively planted soil, including those from *Nocardioide*s, *Ramlibacter*, *Stenotrophomonas*, *Burkholderia*, *Streptomyces*, *Bacillus* and *Rhizobium* (Additional file 1: Table S7), which showed direct or indirect positive correlations with α -terpeneol in the subnetwork and were further selected to verify their correlations. *Nocardioide*s, which showed a direct positive relationship with α -terpeneol (Fig. 6), was significantly promoted by *in vitro* fumigation with α -terpeneol at concentrations of 5, 10 and 20 $\mu\text{L/L}$ (Fig. 7b). However, *in vitro* fumigation with α -terpeneol did not promote the growth of *Ramlibacter* but showed only weak inhibition (Fig. 7b). In addition, a

synergistic effect on growth promotion between *Stenotrophomonas* and *Nocardioide*s, and between *Streptomyces* and *Ramlibacter* was demonstrated when the colonies of two strains got more closer in *in vitro* co-culture (Fig. 8), which was consistent with the correlations in the co-occurrence network (Fig. 6). Furthermore, we found that the isolates from *Nocardioide*s, *Ramlibacter*, *Burkholderia*, *Streptomyces* and *Bacillus* exhibited antagonistic activities against five soil-borne pathogens of *P. notoginseng* (Table 2), which further confirmed the antagonistic activities of those α -terpeneol-enriched PGPRs in the subnetwork (Fig. 6).

Discussion

NPSFs seriously limit the sustainable development of agricultural production. The core factor in NPSF is the build-up of soil-borne pathogens (Huang et al. 2013; Wei et al. 2018; Luo et al. 2019). Many physical or



chemical treatments can completely kill all microorganisms in the soil to eliminate NPSF but usually face the problem of rapid recolonization by soil-borne pathogens (Mendes et al. 2011; Le Cointe et al. 2016; Gurtler 2017; Kumar et al. 2017; Li et al. 2019; Yang et al. 2019). Encouragingly, soil biofumigation is an emerging strategy to alleviate NPSF by targeted antagonism of plant pathogens or to regulate soil microbial communities (Omirou et al. 2011; Nuñez-Zofio et al. 2012; Wang et al. 2014). Here, we found that α -terpineol, a natural volatile monoterpene alcohol released by pine needles, could alleviate the NPSF of *P. notoginseng* by directly or indirectly suppressing the relative abundance of soil-borne fungi (especially Ascomycota), including fungal pathogens, and enriching beneficial bacteria in soil microbial communities.

α -Terpineol fumigation alleviates NPSF by modifying the microbial community

The cultivation of *P. notoginseng* is seriously limited by NPSF, which is reflected in the build-up of soil-borne pathogens and gradually increased infection of the plant

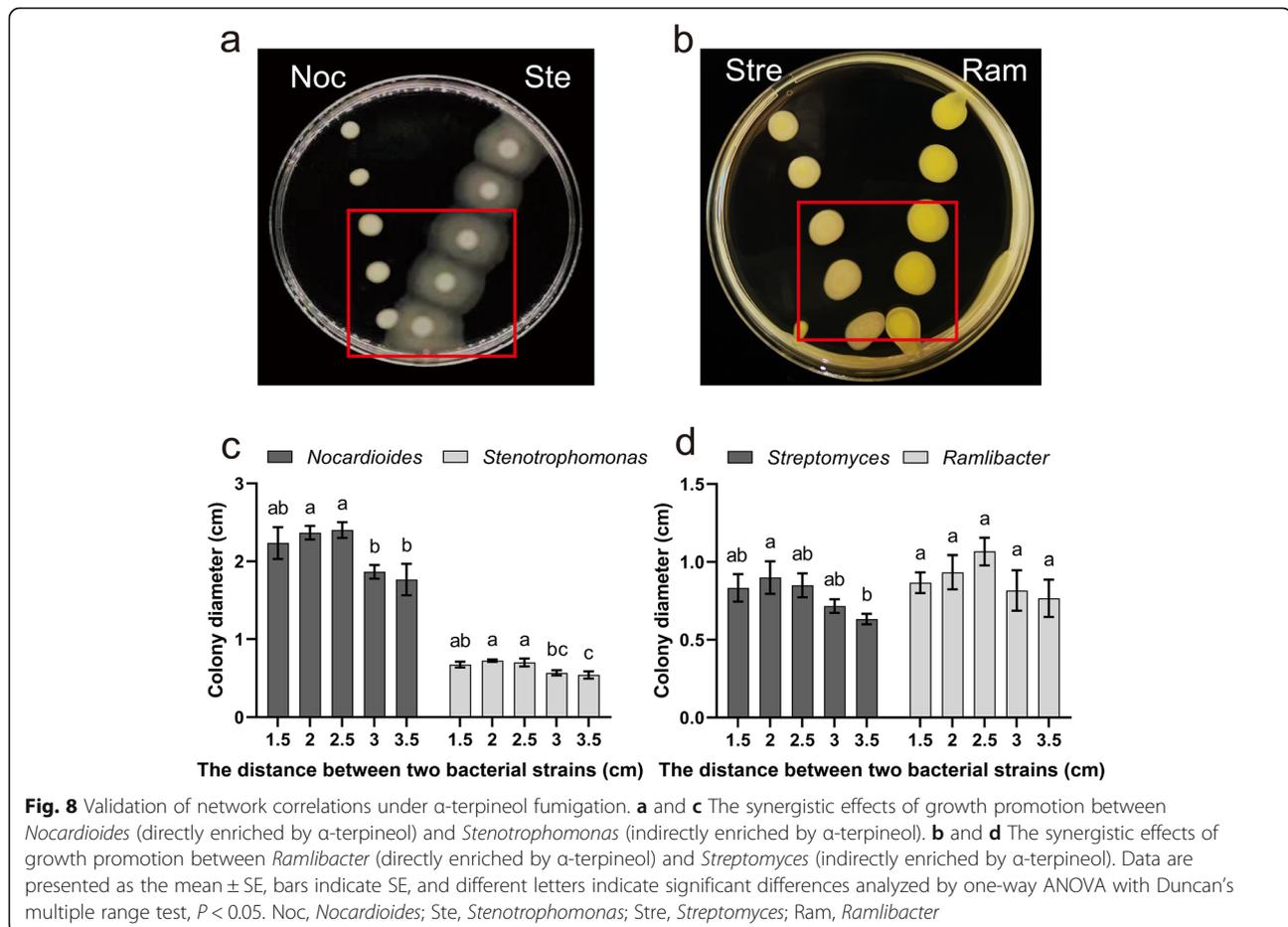


Table 2 Antagonistic effect of bacterial isolates against the soil-borne fungal pathogens of *Panax notoginseng*

Isolate	Inhibition rate (%)				
	<i>A. panax</i>	<i>I. destructans</i>	<i>F. solani</i>	<i>F. oxysporum</i>	<i>P. cactorum</i>
<i>Nocardioides</i>	34.83 ± 0.92	46.83 ± 1.03	28.27 ± 0.62	47.16 ± 0.93	–
<i>Ramlibacter</i>	46.30 ± 0.71	–	43.49 ± 1.73	–	34.32 ± 7.69
<i>Stenotrophomonas</i>	–	46.84 ± 2.83	–	23.23 ± 1.01	–
<i>Burkholderia</i>	42.22 ± 1.65	37.50 ± 7.48	43.43 ± 0.52	51.44 ± 1.55	46.86 ± 1.31
<i>Streptomyces</i>	47.16 ± 1.10	–	–	–	27.85 ± 0.99
<i>Rhizobium</i>	–	–	–	–	–
<i>Bacillus</i>	42.84 ± 1.06	39.89 ± 1.02	47.85 ± 1.44	–	77.70 ± 0.4

A. panax, *Alternaria panax*; *I. destructans*, *Ilyonectria destructans*; *F. oxysporum*, *Fusarium oxysporum*; *F. solani*, *Fusarium solani*; *P. cactorum*, *Phytophthora cactorum*. “–” indicates that there is no antagonistic effect between two strains

by these pathogens (Wei et al. 2018; Yang et al. 2019). Our pot experiments demonstrated that α -terpineol fumigation of soil consecutively planted with *P. notoginseng* could significantly decrease seed decay (Fig. 2). Further study revealed that soil culturable fungi were significantly suppressed by α -terpineol fumigation in a dose-dependent manner, but culturable bacteria were enriched (Fig. 3). Consistently, high-throughput sequencing analysis confirmed that fungi were more sensitive to α -terpineol than bacteria based on the changes in relative abundance (Fig. 4c, d), richness and diversity indices (Additional file 2: Figure S3) and PCoA (Fig. 4a, b). Moreover, *in vitro* fumigation experiments indicated that α -terpineol showed broad-spectrum antifungal activities (Fig. 7a and Additional file 2: Figure S4) but only weak antibacterial activities, even directly promoting the growth of some bacteria, such as *Nocardioides* (Fig. 7b). Thus, these data demonstrated that α -terpineol fumigation could selectively suppress fungi but enrich bacteria to shift unity from a fungal-dominant to a bacterial-dominant soil microbial community. Previous reports have shown that a fungal-dominant microbial community can aggravate the root rot of *P. notoginseng* (Wei et al. 2018; Luo et al. 2019). In contrast, many researchers have reported that an increase in the relative abundance of bacteria, especially antagonistic bacteria, can significantly reduce the harm of pathogens (Latz et al. 2012; Mendes et al. 2018; Wei et al. 2019), even forming disease-suppressive soil to protect plant health (Mendes et al. 2011; Cha et al. 2016; Siegel-Hertz et al. 2018). Likewise, a bacteria-dominant microbial community has advantages for microbial interactions and may favor bacteria-driven soil nutrient cycling (Bahram et al. 2018). Therefore, α -terpineol fumigation could alleviate NPSF by changing soil microbial structure and function. This feature can help to avoid the disadvantages caused by soil chemical fumigation (Ibekwe et al. 2001; Gurtler 2017; Chen et al. 2018). Additionally, the enrichment of PGPRs by α -terpineol fumigation could remove the

barrier of deficient colonization by artificially inoculated PGPRs in the soil (Gómez Expósito et al. 2017).

α -Terpineol fumigation suppresses fungal pathogens in Ascomycota to alleviate NPSF

Several previous studies demonstrated that most soil-borne pathogens, such as *Alternaria*, *Fusarium* and *Cylindrocarpon*, which belong to Ascomycota, could be enriched in the rhizosphere of *P. notoginseng* and ultimately cause severe NPSFs (Miao et al. 2006; Mao et al. 2014; Wei et al. 2018; Luo et al. 2019). Our data indicated that Ascomycota dominated the fungal community in consecutively planted soil but showed a significant dose-dependent decrease when the consecutively planted soil was fumigated with α -terpineol (Fig. 4c). Further network analysis suggested that the nodes belonging to Ascomycota dominated the negative microbial co-occurrence network (Fig. 5c and Table 1). Likewise, keystone taxa are usually regarded as hubs of a network and can be considered drivers of microbiome structure and function (Banerjee et al. 2018; Hartman et al. 2018; Banerjee et al. 2019). In this study, we found that 7 out of 10 keystone taxa of the negative network belonged to Ascomycota (Table 1). These results further indicated that Ascomycota was suppressed by α -terpineol fumigation.

Furthermore, we observed that the potential pathogen *Alternaria* showed a direct negative correlation and the pathogens *Gibberella* and *Phytophthora* exhibited indirect negative correlations with α -terpineol in the network (Fig. 6). The *in vitro* antifungal tests confirmed that α -terpineol could inhibit the growth of five soil-borne pathogens of *P. notoginseng*, four of which belong to Ascomycota (Fig. 7a). In addition, six non-pathogenic fungi belonging to Ascomycota were also inhibited by α -terpineol in a dose-dependent manner (Additional file 2: Figure S4). This was consistent with previous reports that α -terpineol showed antifungal activity against Ascomycota (Hammer et al. 2003; Pinto et al. 2014; Zhou

et al. 2014). Although α -terpineol was previously reported to disrupt the cell membrane integrity of *Geotrichum citriaurantium* (Zhou et al. 2014) and trigger the programmed cell death of *Chlamydomonas reinhardtii* by increasing reactive oxygen species (ROS) (Chen et al. 2019), the antifungal mechanisms of α -terpineol remain worthy of further study.

α -Terpineol directly or indirectly enriches beneficial bacteria to antagonize soil-borne pathogens

With the occurrence of NPSF, the abundance of Acidobacteria, Planctomycetes and Actinobacteria was significantly suppressed in the rhizosphere of *P. notoginseng* (Luo et al. 2019). Here, we found that Acidobacteria, Planctomycetes and Actinobacteria could be enriched by α -terpineol fumigation (Additional file 1: Table S3). Isolates from these phyla are widely used to protect crops from diseases and to decompose cellulose (Loqman et al. 2009; Eichorst et al. 2011; Sathya et al. 2017). Moreover, based on network analysis, we found that a total of 20 genera, mainly Proteobacteria, were significantly enriched by α -terpineol (Fig. 5a). Network analysis also demonstrated that Proteobacteria dominated the positive network, and *Aquicella*, *Cellvibrio* and *Dyella*, which belong to Proteobacteria, were identified as keystone taxa in the microbial network (Table 1).

The subnetwork analysis subsequently showed that α -terpineol not only directly modified bacteria but also indirectly enriched many PGPRs through these directly modified bacteria (Fig. 6). Among these bacteria directly or indirectly modified by α -terpineol, there were many well documented PGPRs, such as *Nocardioidea*, *Pantoea*, *Thiobacillus*, *Cellvibrio*, *Stenotrophomonas*, *Burkholderia*, *Streptomyces*, *Bacillus* and *Rhizobium*, which play important roles as antagonists against pathogens, producing phytohormone auxins, improving soil fertility and so on (El-Azeem et al. 2007; Mishra et al. 2011; Ullah et al. 2013; Pii et al. 2015; Vejan et al. 2016; Besharati 2017) (Fig. 6). Network analysis further suggested that these enriched PGPRs showed potential antagonistic activities, which were reflected in the negative correlations with pathogens in the subnetwork (Fig. 6), and the strong antagonistic activity against pathogens shown by these isolates from *Nocardioidea*, *Ramlibacter*, *Burkholderia*, *Streptomyces* and *Bacillus* subsequently verified these correlations (Table 2).

Hassani et al. (2018) and Durán et al. (2018) reported that microbe-microbe interactions are important for the establishment and maintenance of the host-microbiota balance as well as improving plant host survival. Through the subnetwork analysis, we further found that α -terpineol could modify the assemblages of the soil microbial community to enrich PGPRs by directly or indirectly affecting the interactions of microbes. First, α -

terpineol could directly promote the growth of some PGPRs. This was confirmed by the effect of *in vitro* α -terpineol fumigation on *Nocardioidea*, which showed positive correlations with α -terpineol (Fig. 7b). Second, α -terpineol could affect interkingdom interactions to modify the microbial community. Recent evidence has indicated that microbial interkingdom interactions play critical roles in shaping soil microbial communities (Bahram et al. 2018; D'Souza et al. 2018; Finkel et al. 2019) and can typically be classified as antagonistic, neutral, and beneficial (Little et al. 2008; D'Souza et al. 2018). In this study, we found that *Ramlibacter* showed direct positive correlations with α -terpineol in network analyses (Fig. 6), but α -terpineol *in vitro* fumigation did not show a direct effect on the growth of *Ramlibacter*. Due to the strong antagonistic interactions between *Ramlibacter* and soil-borne fungal pathogens (Table 2), the antagonistic interactions might be weakened, resulting in the enrichment of *Ramlibacter* when the fungi suffered broad-spectrum suppression under α -terpineol fumigation. Likewise, Agler et al. (2016) previously reported that some fungal hub microbes could control the abundance of bacteria by suppressing the growth and diversity of other microbes. However, this relationship still needs to be further verified. Third, α -terpineol could indirectly recruit PGPRs. In this study, we found that although α -terpineol cannot directly modify most PGPRs, V-shaped co-culture experiments showed that microbes that were significantly positively correlated with α -terpineol could promote the growth of many PGPRs (Fig. 8). However, the mechanisms of this synergistic assembly of PGPRs under α -terpineol fumigation need further study. Encouragingly, cross-feeding interactions, in which bacteria frequently exchange metabolites such as vitamins, amino acids, nucleotides or growth factors (D'Souza et al. 2018), provided clues for further verification.

Conclusions

Our results showed that α -terpineol fumigation could alleviate NPSF of *P. notoginseng* by rebalancing soil microbial communities, and this was mainly reflected by the inhibition of soil-borne fungal pathogens and the enrichment of PGPRs. The enrichment of PGPRs exerted strong antagonistic activity to soil-borne pathogens of *P. notoginseng*, which might play an important role in the maintenance of soil health during the growth of *P. notoginseng*. Thus, α -terpineol can be used as a soil biofumigant, as well as a microbial community improver, to reduce the impact of NPSFs in an environmental-friendly mode.

Methods

VOC detection and α -terpineol identification

Fresh pine needles of *P. yunnanensis* were collected from pine trees in Deqin County (Yunnan Province, China,

28°2'5"N, 99°29'22"E, 2860 m), Lijiang City (Yunnan Province, China, 26°57'16"N, 100°3'34"E, 1840 m) and Chuxiong City (Yunnan Province, China, 24°42'37"N, 101°21'17"E, 1640 m). The dried pine needles were made into powder, and VOCs were extracted for 48 h through a "push-pull" system previously reported by Berhal et al. (2017). Briefly, clean air was pushed through active carbon and silica gel into a chamber by an atmospheric sampling pump (QC-1B, Beijing Municipal Institute of Labour Protection, Beijing, China) at an airflow of 500 mL/min. Pine needle powder (150 g) was placed in the chamber, and a trap filter made of glass column and filled with 0.5 g adsorbent material (Porapak™ Q, 80–100 mesh, Waters, USA) was used to capture the VOCs for 48 h at room temperature in a second atmospheric sampling pump. Three replicates per sample collected from different sites were conducted simultaneously. Additionally, the trap filters were desorbed by 5 mL of n-hexane (EMD Millipore, Billerica, MA, USA) and concentrated to 2 mL under a nitrogen stream. One microliter of sample was analyzed by GC-MS (GCMS-QP2010 Ultra, Shimadzu, Kyoto, Japan). In this system, the GC-MS was equipped with an SH-Rxi-5Sil MS (30 m × 0.25 mm × 0.25 μm, Shimadzu, Kyoto, Japan) and GC column with a carrier gas helium flow of 3.0 mL/min, and the temperature program was as follows: start at 40 °C, followed by an increase of 3 °C/min up to 80 °C and then an increase of 5 °C/min from 80 °C to 260 °C, and then maintained for 30 min. The mass spectrum was recorded for the mass range 35–500 m/z and scanned at a rate of 0.3 s. α-Terpeneol was identified by GCMS solutions software (Shimadzu, Kyoto, Japan) based on the comparison of its mass spectra (MS) with the NIST14 (National Institute of Standards and Technology) library. In addition, the synthetic standard sample of α-terpeneol (97%; J&K Scientific Ltd., Beijing, China) was analyzed by GC-MS to further verify this VOC by comparing the MS, RT and RI, which were calculated by GCMS solution software.

Measuring the alleviation of NPSF by α-terpeneol fumigation

Consecutively planted soil is the soil that had been planted with *P. notoginseng* for one or more years and showed replant failure. In this study, the consecutively planted soil with a one-year history of *P. notoginseng* cultivation (pH: 7.306; electrical conductivity: 597 μS/cm; available N: 121.45 mg/kg; available P: 158.36 mg/kg; available K: 685.03 mg/kg; Organic matter: 55.33 g/kg) was collected from Xundian County, Yunnan, China (25°31'01.4"N, 103°16'48.9"E) in 2018 to test the effect of fumigation with α-terpeneol on NPSF. We transferred 2000 g of consecutively planted soil to a sealed plastic box in which a sterilized centrifuge tube lid containing

α-terpeneol was then placed to fumigate the soil at concentrations of 0, 5 and 50 μL/L (volume of pure α-terpeneol /volume of the sealed plastic box). The soil treated by steaming for 15 min at 121 °C was used as a positive control. The fumigation lasted for 4 weeks, during which the α-terpeneol was added once a week and the water holding capacity was kept at 30%. After fumigation, 60 g of fumigated or control soil was transferred to a pot (4.0 × 4.0 × 8.0 cm), and 10 seeds of *P. notoginseng* were sown subsequently. Before sowing, the seeds were surface-disinfected according to Luo's method (Luo et al. 2019). Each treatment included 20 pots and was divided into 5 replicates, and all pots were randomly placed in a greenhouse (25 ± 2 °C, 12 h light/12 h dark) and watered once a week to keep the water holding capacity at 30%. After 50 days, the seed decay rate was calculated as follows:

$$\text{The seed decay rate (\%)} = \frac{\text{Decayed seeds}}{\text{Total seeds in each treatment}} \times 100$$

Measuring the effects of α-terpeneol fumigation on the composition of soil microbiome

In this experiment, we used divided petri plates for the contactless fumigation of soil consecutively planted with *P. notoginseng*. One compartment of this plate was filled with 10 g of the abovementioned consecutive soil, and another compartment held a centrifuge tube lid containing α-terpeneol standard. The plate was sealed with parafilm. The entire experiment lasted for 4 weeks based on a previous study by Yuan et al. (2017). The concentrations of α-terpeneol fumigation were set at 0, 5, 20 and 50 μL/L (volume of pure α-terpeneol/volume of divided petri plates). During the experiment, α-terpeneol was added once a week and the water holding capacity of soil was kept at 30%. All plates, including three replicates of each treatment, were randomly placed in a greenhouse (25 ± 2 °C, 12 h light/12 h dark).

Measuring the effects of α-terpeneol fumigation on culturable fungi and bacteria in fumigated soil

Each treatment, which consisted of 10 g of fumigated soil samples, was divided into two parts: one part was stored at –80 °C for subsequent experiments, and the other 5 g soil sample was used to assess culturable fungi and bacteria on plates as previously reported by Yang et al. (2019). Briefly, a 5 g soil sample in each treatment was added to 45 mL of sterilized water. After 15 min of homogenization, the soil suspension was decimally diluted from 10⁻¹ to 10⁻⁷, and then 100 μL of diluted soil suspension was plated on rose bengal medium (RBM) and nutrient agar (NA) to count fungi and bacteria, respectively (Luo et al. 2019). After incubation at 25 °C for 4–5 days, the colony forming units (CFU) were counted,

and the mean values from the counts of four replicates were obtained. The results were expressed as CFU per gram of dry soil. The experiment was repeated three times.

DNA extraction and sequence analysis

The total DNA of treated soil was extracted from a 0.5 g soil sample by using the Power Soil® DNA Isolation Kit (Mo Bio Laboratories Inc., USA) following the manufacturer's instructions. The quality of the extracted DNA was determined using a spectrophotometer (NanoDrop 2000, Thermo Fisher Scientific, USA). The V4 region of the bacterial 16S rRNA (ribosomal RNA) gene was amplified by primers 515F (5'-GTGCCAGCMGCCGCGG TAA-3')-806R(5'-GGACTACHVGGGTWTCTAAT-3'), and The ITS2 region of the fungal ITS rRNA gene was amplified with primers ITS3_KYO2(5'-GATGAA GAACGYAGYRAA-3')-ITS4(5'-TCCTCCGCTTATTG ATATGC-3')(Zhou et al. 2019). The amplification products were examined, quantified, and further used to generate sequencing libraries with the TruSeq DNA PCR-Free Sample Prep Kit (Illumina, San Diego, USA). Finally, the fungal ITS and bacterial 16S rRNA genes in the total DNA samples were sequenced using the Illumina HiSeq2500 PE250 platform (Rhoin Biosciences Co., Ltd., Chengdu, China). Raw sequence data were spliced by FLASH, and quality was controlled by Trimmomatic (Magoč and Salzberg 2011; Bolger et al. 2014). The chimera was further removed using UCHIME (Edgar et al. 2011). The UPARSE algorithm in Usearch 7.1 (<http://drive5.com/uparse>) was used to cluster the retained effective tags into operational taxonomic units (OTUs) at a 97% similarity level (Edgar 2013). Taxonomy was assigned based on the UNITE database (ITS) and the SILVA database (16S) through the UCLUST method (Edgar 2010). The data for each sample were processed by normalization based on the minimum data in the sample. Additionally, the Chao1 and Simpson indices were calculated to measure the richness and diversity of the microbial community.

Construction and analysis of the microbial network

In this study, all the microbial networks were constructed at the genus level using Pearson correlation analysis. First of all, the relative abundance of different genera after treated with α -terpineol was calculated using Pearson correlation analysis, and only these genera (called 'directly correlated genera') showing statistically significant ($P < 0.05$) linear relationships were selected. To further explore the effects of these directly correlated genera on other microbes, we calculated the Pearson's R between the directly correlated genera and other genera, and only these genera (called 'indirect correlated genera') significantly correlated ($P < 0.05$ and Pearson's $|R| > 0.6$) with the directly correlated genera

were furtherly used to construct network. The networks were then constructed using Cytoscape version 3.7.0 (Shannon et al. 2003) based on the correlations between the directly correlated genera and α -terpineol, as well as between the directly correlated and indirect correlated genera. Finally, the whole co-occurrence network was divided into positive and negative (with α -terpineol) networks according to the correlation between the directly correlated genera and increasing concentrations of α -terpineol. Likewise, a subnetwork highlighting the soil-borne pathogens of *P. notoginseng* and widely reported PGPRs was constructed. All these networks were visualized in Cytoscape version 3.7.0 (Shannon et al. 2003) and Gephi version 0.9.2 (Bastian et al. 2009). The topological properties of the co-occurrence networks were calculated in Cytoscape by the tool NetworkAnalyzer (Assenov et al. 2008). Regarding the topological properties of these networks, the nodes represent the genera of fungi and bacteria, and the links represent the connections between nodes. Additionally, the degree represents the number of links connected to a node. The clustering coefficient shows the connectedness among nodes in the network. In this study, we calculated the degrees of all nodes in two co-occurrence networks and considered the nodes with higher degrees (Top 10) to be keystone taxa (Hartman et al. 2018), which play key roles in driving community composition and function irrespective of their abundance (Banerjee et al. 2018).

Measuring the effect of α -terpineol on soil-borne pathogens

Five soil-borne pathogens infecting *P. notoginseng*, including *A. panax*, *I. destructans*, *F. solani*, *F. oxysporum* and *P. cactorum*, as well as six non-pathogenic fungus that were isolated from consecutively planted soil were selected to validate the antifungal activities of α -terpineol by *in vitro* fumigation. Briefly, a pathogen mycelial block (6 mm in diameter) was placed in the middle of a Petri dish filled with PDA (potato dextrose agar). Then, a sterilized centrifuge tube lid containing α -terpineol was placed in the bottom of an inverted Petri dish sealed with parafilm. After incubation at 28 °C for 6–7 days, the mycelial growth of the pathogen was determined by measuring the colony diameter. Three replicates per treatment were performed with concentrations of 0, 10, 50, 100, 200, 300 and 500 $\mu\text{L/L}$ (volume of pure α -terpineol/volume of petri plates). The growth inhibition rate was calculated as follows:

$$\begin{aligned} \text{The growth inhibition rate (\%)} \\ = \left[\frac{(\text{Radial growth of control} - \text{Radial growth of treated sample})}{(\text{Radial growth of control})} \right] \times 100 \end{aligned}$$

The half maximal (50%) effective concentrations (EC_{50}) were determined according to Förster et al. (2004).

Measuring the effect of α -terpineol on PGPRs

To better evaluate the effects of α -terpineol on PGPRs, we first isolated bacteria from consecutively planted soil by culturomics with different media: nutrient agar (NA), lysogeny broth (LB), tryptic soy agar (TSA), Reasoner's 2A agar (R2A), and VL55 (Nguyen and Kim 2017). All the isolates were molecularly identified by 16S rDNA amplification (Cai et al. 2012), but only those belonging to widely reported PGPRs and showing direct or indirect correlations with α -terpineol in the subnetwork were selected for further experiments.

First, the isolates showing direct positive correlations with α -terpineol in the network were selected, and the growth promotion effects of α -terpineol on them were validated by *in vitro* fumigation. We used soft agar LB medium (only agar reduced to 0.25%) to measure the expansion speed based on the method by Cremer et al. (2019). Briefly, 1 μ L (10^6 CFU/mL) of isolate suspension was inoculated in the middle of the soft agar, and the fumigation method was the same as for fungal *in vitro* fumigation, but the concentrations of α -terpineol were reduced to 0, 5, 10, 20 and 50 μ L/L. Three replicates per treatment were performed. The growth inhibition rate was calculated as:

$$\text{The growth inhibition rate (\%)} = \left[\frac{(\text{Colony diameter of treated sample} - \text{Colony diameter of control})}{(\text{Colony diameter of control})} \right] \times 100$$

Second, we used V-shaped co-culture experiments to validate the indirect enrichment effects of α -terpineol on PGPRs. The synergistic effects of growth promotion between bacteria directly enriched by α -terpineol and those indirectly were determined based on the method by Berendsen et al. (2018). Briefly, 1 μ L of suspension (10^6 CFU/mL) of each of these directly and indirectly enriched bacteria was inoculated five times in a diagonal row of the abovementioned soft agar to create a V-shape of increasingly closer inoculation sites (the distance of two strains was 1.5, 2.0, 2.5, 3.0 and 3.5 cm). Then, the colony diameters were determined.

Measuring the antagonistic activity of bacteria against soil-borne pathogens

In this experiment, a pathogen mycelial block (6 mm in diameter) was placed in the middle of the Petri dish. Then, bacterial suspensions of isolated PGPRs were placed at four sites with the same distance (25 mm) around the pathogen mycelium block. Pathogens grown alone on PDA plate without PGPR inoculation were used as controls. After 5 days of incubation, the inhibition rates of isolated bacteria on fungal pathogens were calculated as follows:

$$\text{Inhibition rate (\%)} = \left[\frac{(\text{Diameter of control} - \text{Antimicrobial band width of treated sample})}{(\text{Diameter of control})} \right] \times 100$$

Statistical analysis

IBM SPSS Statistics version 25 (SPSS Inc., Chicago, Illinois, USA) was used for general statistical analyses. One-way analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) were used to analyze the mean separation among treatments. Pearson's correlation coefficient was employed to correlate the relative abundance of microbes at the genus level with increasing concentrations of α -terpineol. The Chao1 and Simpson indices were calculated using R software (vegan package). Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was performed by R software (APE package). Some data analyses are described in the corresponding sections above.

Abbreviations

CFU: Colony forming units; EC₅₀: Effective concentration for 50% inhibition; GC-MS: Gas chromatography-mass spectrometry; LB: Lysogeny broth; MS: Mass spectra; NA: Nutrient agar; NIST: National Institute of Standards and Technology; NPSFs: Negative plant-soil feedbacks; OTU: Operational taxonomic units; PCoA: Principal coordinates analysis; PDA: Potato dextrose agar; PGPRs: Plant growth-promoting rhizobacteria; PSFs: Plant-soil feedbacks; R2A: Reasoner's 2A agar; RI: Retention index; RT: Retention time; SAR: Systemic acquired resistance; TSA: Tryptic soy agar; VOC: Volatile organic compounds

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-021-00090-1>.

Additional file 1: Table S1. Processed sample data used to analyze the fungal community. **Table S2.** Processed sample data used to analyze the bacterial community. **Table S3.** Correlation between α -terpineol and the relative abundance of fungi or bacteria at the phylum level. **Table S4.** Widely reported PGPRs in agricultural system. **Table S5.** Twenty-three fungal isolates from *Panax notoginseng* field soil. **Table S6.** One hundred and eighty-nine bacterial isolates from *Panax notoginseng* field soil. **Table S7.** Bacterial isolates used for co-culture and antagonistic experiments.

Additional file 2: Figure S1. Heat map illustrating that the high abundance of *Escherichia-Shigella* overshadowed the remaining bacterial microbial community at the genus level. **Figure S2.** Effect of the removal of *Escherichia-Shigella* reads on bacterial community beta diversity. **Figure S3.** Effects of 4 weeks of α -terpineol fumigation on the alpha-diversity indices of fungal and bacterial communities in soil consecutively planted with *Panax notoginseng*. **Figure S4.** Effects of α -terpineol on the growth of non-pathogenic fungus of *Panax notoginseng*.

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Authors' contributions

HH, SZ, YYZ and XH conceived and designed the experiments. CY, YL, JZ, TL, YJZ and CG carried out the experiments. CY analyzed the data. CY, YL, HH, MY and SZ wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Raw sequencing data are accessible under NCBI BioProject ID: PRJNA639436.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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