1 **Composition variation of agarwood-associated microbial communities from** 2 *Aquilaria sinensis*

3 Qigui Mo ¹, Chenyang Fan ¹, Gao Zhou ¹, Huiying Fu ¹, Youwei Wang ^{1,2*}

4 **Running Head: Endophytic bacteria and fungi of agarwood**

5 **ABSTRACT** Agarwood, derived from *Aquilaria sinensis* and *Aquilaria malaccensis*, is of medicinal
6 and ecological value and religious importance as incense. The existing imbalance between short supply
7 and increasing demand of this product remains to be solved. Thus, the biologically artificial agarwood-
8 inducing methods commonly called whole-tree agarwood-inducing techniques (agar-wit) have been
9 established to dramatically improve agarwood yield within a short period. However, several studies
10 reported a lower content of ethanol-soluble extractive in the agar-wit agarwood than in the natural
11 agarwood. To further understand the role of microorganisms in agarwood formation, we investigated
12 and contrasted the endophytic bacteria and fungi between different types of agarwood from *A. sinensis*
13 through high-throughput sequencing. Results showed that the same dominant phyla of bacteria
14 consisting of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* were shared by the natural agarwood
15 and agar-wit agarwood. Meanwhile, *Ascomycota* and *Basidiomycota* constituted the similar dominant
16 fungal phyla of these two kinds of agarwood. However, the principal microbial communities at the
17 genus or order level evidently varied from natural agarwood to agar-wit agarwood. Moreover, the
18 bacterial communities are closely connected with terpenoid and carbohydrate metabolism, which
19 indicated that the bacterial communities also play a vital role in agarwood formation. In conclusion, the
20 higher concentrated abundance of the dominant microbial communities in agar-wit agarwood than in
21 natural agarwood may promote agarwood formation, however, the low evenness of microbial
22 communities also lowers the content of ethanol-soluble extractive.

23 **KEYWORDS** natural agarwood, agar-wit agarwood, high-throughput sequencing, bacterial diversity,
24 fungal diversity

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25 **IMPORTANCE** Agarwood has become an indispensable product in modern life because of medicinal
 26 value, ecological and religious importance as incense. Nevertheless, the enormous demand for
 27 agarwood markedly exceeds the supply because of the dramatically declining population of genus
 28 *Aquilaria*. Agarwood formation occurring slowly and infrequently in a natural environment, so various
 29 artificial techniques were developed to promote the formation of agarwood, such as the physical
 30 methods and chemical methods. However, these techniques still are insufficient to compensate for the
 31 agarwood shortage. In this case, a novel biological method called the whole-tree agarwood-inducing
 32 technique (Agar-wit) induces agarwood production. However, several studies have shown that
 33 agarwood harvested from biological technology contains lower content of ethanol-soluble extractive
 34 compared with natural agarwood. So to further expose and understand the endophytic bacteria and
 35 fungi in agarwood formation is important for the improvement of biological method.

36 **INTRODUCTION**

37 *Aquilaria sinensis* (Lour.) Gilg (*Thymelaeaceae*) is mainly distributed in the provinces of Hainan,
 38 Guangdong, Fujian, and Yunnan in China (1, 2), and *Aquilaria malaccensis* Lam is native to Malaysia
 39 (3). These plants are sources of agarwood, which is the dark resinous wood harvested from the tree
 40 (China Pharmacopoeia 2015, first section). Agarwood is highly prized for its aphrodisiac, sedative,
 41 cardiotonic, and carminative effects and its ability to relieve gastric problems, coughs, rheumatism, and
 42 fever (4). Agarwood (Chen Xiang in China), used as a traditional Chinese medicine to dispel damp
 43 toxin, was first documented in Mingyi Bielu in the Wei and Jin Dynasties. Traditional use was
 44 complementally recorded in Bencao Gangmu in the Ming Dynasties. Recently, many chemical
 45 ingredients of agarwood have revealed significant anticancer (5) and anti-inflammatory activities (6).
 46 Moreover, agarwood has been used as incense for centuries in Buddhist, Hindi, and Islamic ceremonies
 47 (4). DingWei, a famous writer in the Song Dynasty, provided crucial contributions to the development
 48 of agarwood as incense. In Tianxiang Zhuan, he was the first to classify agarwood into four grades and
 49 12 shape categories depending on the fragrance, appearance, and formation. In a word, agarwood has
 50 become an indispensable product in modern life.

51 Nevertheless, the enormous demand for agarwood markedly exceeds its supply because of the
 52 dramatically declining population of the genus *Aquilaria* caused by illegal overexploitation (7). This
 53 genus has been listed in Appendix II of the Convention on International Trade in Endangered Species

of Wild Fauna and Flora since 2004 (1). The factors mentioned above explain the price inflation of agarwood products to US\$ 100,000/kg for superior, pure material (4). Natural agarwood forms around trunk wounds, particularly external wounds caused by lightning strikes, animal attack, which easily induce the wounds to infect with microorganisms (8). As agarwood formation occurs slowly and infrequently in the natural environment, various artificial techniques have been developed to promote agarwood formation. Such techniques include physical methods where tree trunks and branches are wounded using axes, fire, knives, or nails (9) and chemical methods where the wounds are intentionally treated with some chemical reagents initially (e.g., inorganic salt, acids, and phytohormone) (10). However, these techniques are insufficient to compensate for the agarwood shortage. In this case, a novel biological method called the whole-tree agarwood-inducing technique (agar-wit) induces agarwood production by inoculating certain elicitors into the xylem part of *Aquilaria* trees through simple transfusion sets (12). The prepared inducer is then transported throughout the whole tree gradually by transpiration force and initiates a series of defensive responses to produce resin (12). A remarkable biological inoculation is the fungal inoculum, which is mainly fermentation liquid of fungi, such as *Menanotus flavolives* (13), *Botryosphaeria dothidea* (14), and *Lasiodiplodia theobromae* (12). As reported, such inoculum is capable of stimulating agarwood formation. Given the fungal mobility and plant transpiration, agarwood may form on the trunk and branches of the entire tree, causing a far higher production of agarwood at a lower cost of labor and time than that induced by previous artificial techniques. However, several studies have shown that agarwood harvested from biological technology contains lower content of ethanol-soluble extractive compared with natural agarwood (15-17). Zou (18) considered the possible role of endophytic bacteria in agarwood formation, but failed to determine the bacterial community structure by PCR–DGGE methods. Thus, the bacterial and fungal communities of agarwood from *A. sinensis* must be investigated to refine the current agar-wit technique. In this study, the major bacterial and fungal communities of natural agarwood and agar-wit agarwood were investigated using high-throughput sequencing without the prepared isolation and cultures. The variation of dominant microbial communities between these two agarwood types was analyzed. Functional profiles were also used to investigate the role of bacterial communities in the metabolic activity of agarwood formation. Finally, the relationship between the variation of dominant microbial communities and agarwood types was characterized.

RESULTS

General analyses. After data filtration, a total of 64474 and 37850 high-quality sequences of all samples were obtained for the fungal primer SSU0817F/1196R and bacterial primers 799F/1392R and 799F/1193R, respectively. Then, these effective sequences were clustered to OTUs with 97% similarity, which is a common parameter for describing microbial community structure (19, 20). After the chimeric, mitochondrial, and chloroplast sequences were removed, the OTU table generated 30 OTUs and 185 OTUs for fungal and bacterial communities, respectively. These OTU tables were used for downstream analyses.

First, rarefaction curves were applied to evaluate and compare data from the current study (Fig. S1). With increasing read number, the curves tended to stabilize and be saturated for both bacterial and fungal taxonomies; this finding suggested that the sequenced numbers were adequate to reflect the vast majority of microbial types, and the structures of bacterial and fungal communities associated with different agarwood samples could be reasonably characterized by a sufficient saturated OTU number (21).

Bacterial and fungal diversity analyses. Chao and Shannon indices were related to microbial communities' richness and diversity and are usually used to preliminarily compare the different types of samples (22-24). In our study, the Chao and Shannon indices of bacteria (Fig. 1a and 1b) in natural agarwood samples were higher than those in agar-wit samples, and the same trend was found in fungal communities (Fig. 1c and 1d). These results indicated that the microbial types in natural agarwood, including bacteria and fungi, were more abundant and complicated than those in the agar-wit samples. In both natural agarwood and agar-wit samples, the values of Chao and Shannon indices of bacterial types (Fig. 1a and 1b) were significantly higher than those of fungal types (Fig. 1c and 1d), which suggested that the bacterial communities showed higher abundance than the fungal communities in agarwood samples from two different production methods.

Cross-OTU comparisons in natural agarwood and agar-wit samples. To determine the relation between these two kinds of samples, we conducted cross-OTU comparison by using Venn diagrams (25). The overlaps were present in two different types of microorganisms between natural and agar-wit agarwood (Fig. 2a and 2b). The bacterial and fungal OTUs enriched in the natural agarwood successfully colonized the agar-wit agarwood, as 77 out of 185 OTUs of bacteria included in natural agarwood were also enriched in the agar-wit agarwood (Fig. 2a), and the fungal OTUs in the agar-wit agarwood were almost completely replicated from natural agarwood (Fig. 2b). These results indicated

114 that the bacterial species in agarwood obtained from whole-tree agarwood-induction technology were
115 stabilized and only slightly varied relative to that in natural agarwood.

116 A total of 77 and 17 OTUs were clustered to the common region, which were all shared by two
117 different agarwood samples for bacterial and fungal types, respectively (Fig. 2c and 2d). A total of 77
118 OTUs for bacteria mainly consisted of *Proteobacteria* and *Actinobacteria* (Fig. 3a), and 17 OTUs for
119 fungi were mainly composed of *Ascomycota* (Fig. 3b). These same OTUs were enriched in two
120 different agarwood samples with different abundances. The majority of *Proteobacteria* bacterial OTUs
121 were allocated to *Burkholderiaceae* (15.09%) and *Sphingomonadaceae* (13.47%), whereas most of the
122 *Actinobacteria* bacterial OTUs corresponded to *Microbacteriaceae* (9.18%) and *Solirubrobacterales*
123 (9.14%). The *Ascomycota* fungal OTUs contained *Coniochaetaceae* (29.92%) and *Sordariomycetes*
124 (29.79%, OTUs 36 and 28). These results indicated that the shared microbial communities by natural
125 agarwood and agar-wit agarwood were concentrated on several types.

126 **Microbial community composition in natural agarwood and agar-wit agarwood.** In this study, we
127 investigated the bacteria at different taxonomic levels, and taxonomies with < 1% abundance were
128 defined as others (Fig. 3a and 3c). The dominant phyla consisted of *Proteobacteria*, *Actinobacteria*,
129 and *Acidobacteria*, which constituted the main sections of the bacterial community structure of natural
130 agarwood and agar-wit agarwood (Fig. 3a). The agar-wit technology distinctly caused an increasing
131 ratio of *Proteobacteria* in the agar-wit agarwood from 47.87% to 64.04% compared with natural
132 agarwood. Inversely, the relative abundance of *Actinobacteria* decreased from 44.08% of natural
133 agarwood to 30.06% of agar-wit agarwood and showed a reverse trend (Fig. 3a). Similar to
134 *Arabidopsis* (26) and rice (25), an increased pool of *Proteobacteria* was found in the bacterial
135 communities. However, the difference was that the bacterial community composition was simpler in
136 agarwood, and the three bacterial phyla above achieved a total relative abundance of more than 97.60%.
137 The simpler bacterial community composition of agarwood might be due to the lack of soil
138 microorganisms that could influence each other directly compared with *Arabidopsis* and rice. More
139 detailed variation in relative phylum abundance between two different samples could be embodied by a
140 more specific order level (Fig. 3c). Compared with natural agarwood, the substantially increasing ratio
141 of *Burkholderiales* and *Sphingomonadales* and the sharply decreasing ratio of *Solirubrobacterales* in
142 agar-wit agarwood samples were the main reasons for the change in the phyla of *Proteobacteria* and
143 *Actinobacteria* (Fig. 3c). Interestingly, *Xanthomonadales*, which belongs to the phylum *Proteobacteria*,

144 sharply decreased in agar-wit agarwood than in natural agarwood, which was not observed with the
145 phylum *Proteobacteria*. Similarly, the variation tendency of *Micrococcales* was vastly increasing and
146 thus opposite to that of phylum *Actinobacteria* (Fig. 3c). The variation in relative abundance of
147 *Xanthomonadales* and *Micrococcales* may be due to the cooperation and competition among the
148 different microbial types, which have been thoroughly investigated and utilized (27, 28). More details
149 were showed in Table S1. 65.95% OTUs were assigned to genus or species.

150 At present, fungi are considered as the most important microbial factor promoting agarwood
151 formation. Therefore, studies on fungal communities existing in the resinous region of *A. sinensis*
152 emerged in succession over the years (14, 18, 29, 30). In our study, high-throughput sequencing
153 without the prepared isolation and cultures was applied to characterize the fungal community
154 composition in natural agarwood and agar-wit agarwood. The chloroplast, *Amoebozoa* OTUs, and other
155 not belonged to fungi were filtrated, and then 30 OTUs, which included 1 singleton, were obtained.
156 Based on the results, *Ascomycota* and *Basidiomycota* constituted the main fungal community
157 composition with a total relative abundance of more than 99.89% (Fig. 3a and 3b). Consistent with
158 Premalatha's (30) report, the phylum *Ascomycota* occupied the majority of sequence numbers with
159 78.55% relative abundance in natural agarwood and 91.68% relative abundance in agar-wit agarwood
160 (Fig. 3b). On the contrary, the relative abundance of *Basidiomycota* was 21.34% in natural agarwood,
161 which sharply decreased to 8.32% in agar-wit agarwood (Fig. 3b). At the taxonomic order level, the
162 proportion of phylum *Ascomycota* in agar-wit agarwood indicated a rise relative to natural agarwood
163 owing to the increasing percentage of *Sordariales* and another unclassified order. Actually, these two
164 fungal types on the order level belonged to the same class, *Sordariomycetes* (Fig. 3d). Similar to other
165 bacterial communities, the antagonism could manage the relative abundance of some fungal types. The
166 sharply decreasing *Capnodiales* ratio in agar-wit agarwood was mainly attributed to the lost vivosphere,
167 which was compressed by other dominant fungal types. The final taxons of all fungal OTUs were
168 showed in Table 1. Only 8 OTUs were assignable to genus or species. To supplement this, 10 fungal
169 strains were isolated and identified, and all strains were assigned to genus or species. 6 strains belonged
170 to *Sordariomycetes* (Table S2), which also were the most abundant of microbial types in the culture-
171 independent approach (Fig. S2). *L. theobromae*, a strain usually used in whole-tree agarwood-inducing
172 technique, was isolated from both the natural agarwood and agar-wit agarwood.

173 **Comparative analyses between natural agarwood and agar-wit agarwood.** In the heatmap of
174 bacterial and fungal distributions, we used color intensity to compare the top 50 most abundant genera
175 present in the natural agarwood and agar-wit agarwood (Fig. 4). Lower evenness was found in the
176 bacterial communities of the agar-wit agarwood than in the natural agarwood (Fig. 4a). The bacterial
177 types in the agar-wit agarwood focused on several genus levels, such as *Gryllotalpicola*, *Burkholderia*-
178 *Paraburkholderia*, *Sphingomonas*, and *Acidothermus* (Fig. 4a). A same trend was found in the fungal
179 communities of the natural and agar-wit agarwood (Fig. 4b). In particular, the distribution revealed a
180 highly concentrated relative abundance of *Sordariomycetes* (Fig. S3).

181 The highly concentrated relative abundance of some microbial communities caused variation in the
182 dominant microbial communities between natural agarwood and agar-wit agarwood (Fig. 5). The main
183 dominant bacterial communities at the genus level contained *Jatrophihabitans*, *Acidibacter*, and an
184 unclassified genus belonging to the family *Elev-16S-1332* in natural agarwood (Fig. 5a and Fig. S4).
185 However, the highly abundant population was changed to *Burkholderia-Paraburkholderia*,
186 *Sphingomonas*, *Gryllotalpicola*, and *Acidothermus* in agar-wit agarwood. Actually, the relative
187 abundance of the bacterial communities at the genus level tended to be thoroughly distributed in natural
188 agarwood, which showed a higher evenness in bacterial communities than that in agar-wit agarwood. A
189 variation in the dominant microbial communities was also found within fungal types on the order level
190 between natural agarwood and agar-wit agarwood (Fig. 5b). The dominant fungal communities
191 changed from *Sordariomycetes*, *Agaricomycetes*, and *Dothideomycetes* in natural agarwood to
192 *Sordariomycetes* in agar-wit agarwood.

193 **Bacterial functional profile.** The PICRUSt tool has been used to predict the functional profile of the
194 16S rRNA gene and achieves a high correspondence with the reference genome across several
195 microbial types (31, 32). This method thoroughly compensates for the weakness of 16S rRNA, which
196 cannot directly provide a functional profile (31). By annotating the Cluster of Orthologous Group
197 (COG) function, we obtained 25 functional classifications, in which general function prediction only
198 and function unknown were the two largest clusters. This result was very similar to a previous study
199 (33). After removing the two above-mentioned functional classifications that tentatively held no
200 connection with agarwood formation, we compared the top 18 most abundant functional classifications
201 (Fig. 6a). As a result, two annotated functions related to agarwood formation, “Carbohydrate transport
202 and metabolism” and “Secondary metabolites biosynthesis, transport and catabolism,” shared a high

relative abundance in the agarwood samples. These two metabolic activities were more active in agar-wit agarwood than in natural agarwood (Fig. 6a). After annotating the Kyoto Encyclopedia of Genes and Genomes (KEGG) function, we obtained above 52.64% relative abundance of metabolic pathways (Fig. 6b). Terpenoid and polyketide metabolism is an important biosynthetic pathway of sesquiterpenes in this study, and besides, carbohydrate metabolism also showed a prominent abundance in two types of agarwood (Fig. 6c). Most of the COG and KEGG functions were higher in agar-wit agarwood than in natural agarwood probably because of the variation in the dominant bacterial communities.

DISCUSSION

Agarwood are more and more popular in our daily life as medicine and incense nowadays. Majority of techniques, mainly including physical, chemical, and biological methods have been established to promote agarwood yield (9-11). Expressly, the biological technique, which mainly inoculated fungal fermentation liquid into *A. sinensis* trees, has been considered as the most promising method to obtain a far higher production of agarwood at a lower cost of labor and time (12-14). So previous studies on microbiomes from *A. sinensis* mainly focused on fungal communities across the white wood and resinous region (14, 29, 30). In the present study, we successfully characterized the bacterial and fungal community structure in the natural and agar-wit agarwood through high-throughput sequencing. We also found that bacterial communities were deeply involved in agarwood formation.

Actually, bacterial communities are drew less attention compared to fungal communities in agarwood formation. Premalatha and Kalra (30), who proposed that pathogenic fungi are the primary causative factor in agarwood formation, attempted to investigate the impact of endophytic fungi on the resinous and white wood of *A. malaccensis*. Zou (18) considered that endophytic fungi and bacteria were involved in agarwood formation, but failed to reveal the bacterial community structure by PCR-DGGE methods. In this study, we found the higher bacterial communities' richness and diversity in both types of agarwood than fungal communities (Fig. 1). Remarkably, we detected a high bacterial pool from *A. sinensis* by high-throughput sequencing, which was typically ignored in the past.

The diversity of microbial communities between the natural and agar-wit agarwood were different in current study. The microbial communities richness and diversity analyses all showed the higher values in the natural agarwood than agar-wit agarwood (Fig. 1). Venn diagrams indicated that the microbial species existing in agarwood obtained from whole-tree agarwood-induction technology were relatively

fixed and only slightly changed relative to that in the natural agarwood (Fig. 2). Although Zou (18) considered that fungal species were significantly abundant in agarwood samples compared with white wood, other sufficient reports shared different ideas. Mohamed (29) found no difference in fungal species between agarwood and white wood. Similarly, Tian et al (14) reported that most of the fungi on the genus level colonized the white wood from resinous sections. Combined with our results, we boldly speculate that the methods to promote agarwood formation, physical, chemical, and microbial infection methods, enhanced the abundance of key microbial species rather than transforming the microbial community structure.

Fungal infection technology promoting agarwood formation is becoming increasingly developed, but this method still has a noticeable deficiency. Several studies proved that the content of ethanol-soluble extractive, a crucial indicator in evaluating agarwood product quality in the Chinese Pharmacopeia (2015), was lower in the agarwood product from agar-wit technology than in the natural agarwood samples (15-17). Therefore, the role of bacterial infection in agarwood formation is increasingly being valued. We successfully characterize the bacterial community composition of agarwood products through high-throughput sequencing, an advanced method that allows the investigation of microbial types without the prepared isolation and cultures (25, 34). Although the richness and diversity of microbial communities and cross-OUT comparison analyses showed the difference of microbial communities in the natural and aga-wit agarwood, these two types of samples shared the same dominant phyla for both bacterial and fungal communities (Fig. 3a and 3b). On order level, the abundances of most microbial categories were different, which might cause the different values of microbial communities' richness and diversity.

In particular, unlike in most previous studies, the comparative analysis of *A. sinensis* between the white wood and the resinous region was performed to determine the most important microbial communities in agarwood formation (14, 18, 29, 30). In our study, we compared the bacterial and fungal types between the natural agarwood and agar-wit agarwood and found a variation in the dominant microbial communities between these two kinds of agarwood samples. In the cross-OTU comparative analysis, we summarized that the bacterial and fungal species enriched in the natural agarwood successfully colonized the agar-wit agarwood. This result indicated no difference in microbial types between these two kinds of agarwood. In the current study, the primer

SSU0817F/1196R, which showed a fungal-specific characteristic, was employed to accurately characterize the fungal communities without any other false-positive results (35). However, the taxonomic identification from this primer was restricted (35). In our study, most of the fungal communities were characterized on the order level or above (Fig. S3). Actually, next-generation sequencing technology (NGS) have many constrains (36). The fungal DNA may not be recovered from all genotypes in the culture-independent approaches (36). Besides, PCR amplification steps may prefer to the bulk DNA extracts, and hinder the identification of some other genotypes (37). Finally, fungal sequences obtained from the culture-independent approaches are short and variable, which restrict the assignment of fungal sequences to genus or species (38). So the culture-independent methods are usually used to reflect the microbial communities, and the culture-dependent methods are used to identify the microbial taxons. These might be the reasons why no genus or species overlapped between the culture-dependent and culture-independent approaches in this study. Despite all these shortcomings, a lower evenness of fungal communities was found in the agar-wit agarwood than in natural agarwood at the genus level (Fig. 4b). Bacterial communities also showed the lower evenness in agar-wit agarwood and focused on several genus levels (Fig. 4a). The lower evenness and higher concentrated relative abundance of microbial communities resulted in variation of the dominant microbial communities between natural agarwood and agar-wit agarwood (Fig. 5). Given the above results, the highly concentrated relative abundance of the dominant microbial communities in agar-wit agarwood might be a key factor in promoting agarwood formation relative to that in natural agarwood. However, the decreased evenness of microbial communities also caused the lower content of ethanol-soluble extractives.

The role of bacterial communities in agarwood formation is unknown, we proved that bacterial communities were deeply involved in agarwood formation in current study. The annotated COG and KEGG functions showed that bacterial communities shared the abundant functions of terpenoid and carbohydrate metabolism in natural and aga-wit agarwood. Sesquiterpenes comprise the main component of agarwood (39). Sesquiterpene synthesis begins with some complex pathways from glycolysis (40, 41). Thus, carbohydrate metabolism with high relative abundance can provide a material for sesquiterpene synthesis in agarwood formation (Fig. 6c). The increased level of terpenoid and carbohydrate metabolism benefits agarwood formation in agar-wit agarwood samples. Several bacteria have been reported to participate in the synthesis of terpenes (42-45). In this study, an

Mycobacterium OTU and three *Streptomyces* OTUs were identified (Table S1), which might be the probable contributors for the COG and KEGG functions of terpenoid and carbohydrate metabolism. In this section, the results of the functional profile of the 16S rDNA gene demonstrated that bacterial communities also played a critical role in agarwood formation.

In summary, we successfully characterized the bacterial and fungal community composition in natural agarwood and agar-wit agarwood. In these two kinds of agarwood products, the dominant phyla of bacteria consisted of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria*. Meanwhile, the same predominant fungal phyla included *Ascomycota* and *Basidiomycota*. The variation of the dominant microbial communities between natural agarwood and agar-wit agarwood has been described at the taxonomic level of genera or order. The functional profile predicted using the PICRUSt tool indicated that bacterial communities were deeply involved in agarwood formation. We inferred that the highly concentrated relative abundance of the dominant microbial communities in agar-wit agarwood can more effectively promote agarwood formation than that in natural agarwood. However, the low evenness of microbial communities also results in the low content of ethanol-soluble extractive. We believe that this study will make it easier to understand the role of bacterial communities in agarwood formation.

MATERIALS AND METHODS

Agarwood samples. Two kinds of agarwood samples were collected from mature *A. sinensis* (Lour.) Gilg trees in Haikou, China (E109°27'26.08", N19°42'20.02"), in June 24, 2017. Natural agarwood samples were cut off at 2–3 cm thickness from the exposed section where agarwood formed naturally. Agar-wit samples were then collected from the bottom of the trunk into which fungal inducers were injected 24 months earlier. All samples were cut with a sterilized knife or saw, placed in clean sealable bags immediately, and transported back to the laboratory under 4 °C. Excess white wood was removed, and the remaining agarwood was surface sterilized with 75% ethanol and finally stored in an ultra-low-temperature freezer (Haier biomedical, Qingdao, China) for endophytic microbial diversity analysis.

DNA extraction. A series of experiments, including DNA extraction, PCR amplification, and pyrosequencing, were carried out on the agarwood samples by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Briefly, agarwood samples were separately milled into powder under liquid nitrogen. Total DNA was extracted from 0.5 g powdered agarwood in accordance with a previously

described CTAB (Cetyltrimethylammonium Ammonium Bromide) method (30). Total DNA was quantified by agarose gel electrophoresis and then used for PCR amplification.

PCR amplification of the V5–V7 region of the fungal 18S and bacterial 16S rRNA genes. The V5–V7 region of the 18S rRNA fungal-specific primers (SSU0817F: 5'-TTAGCATGGAATAATRRAATAGGA-3' and 1196R: 5'-TCTGGACCTGGTGAGTTTCC-3') were designed for endophytic fungal diversity analysis (35). Meanwhile, to accurately assess the endophytic bacterial community, two sets of amplification primers were designed. The first one was 799F/1392R (799F: 5'-AACMGGATTAGATACCKG-3' and 1392R: 5'-ACGGGCGGTGTGTRC-3'), and the second was 799F/1193R (799F: 5'-AACMGGATTAGATACCKG-3' and 1193R: 5'-ACGTCATCCCCACCTTCC-3') (26, 45, 46). PCR was performed on ABI GeneAmp® 9700 (Applied Biosystems, Foster City, USA) with the following conditions: 2.5 mM dNTPs, 5 µM forward and reverse primers, and 0.4 µL FastPfu polymerase. The cycling process proceeded as follows: 3 min for 95 °C, 27 cycles at the same temperature for 30 s, annealing at 55 °C for 30 s, 72 °C for 15 s, and then extension at 72 °C for 10 min.

Illumina MiSeq sequencing and data analysis. PCR amplification products of the V5–V7 region of fungal 18S and bacterial 16S rRNA genes were sequenced by Illumina MiSeq sequencer. The Illumina raw sequences were filtered by QIIME (47). Specific barcodes were set to assign the different agarwood samples (48). Chimeric sequences were checked and removed. The taxonomic levels of the bacteria and fungi in this study depended on the OTUs (Operational taxonomic units) at 97% similarity, which was defined on the basis of trimmed sequences. These OTUs were assigned based on the Silva database (<http://www.arb-silva.de>). The abundance of each OTU in tables described the level of fungal and bacterial phylotypes in different samples. To determine the most representative microbial community, mitochondrial and chloroplast OTUs, as well as those with < 0.1% abundance in different samples, were filtered manually (49). Meanwhile, OTUs were rarefied to the lowest sequence number. The simplified OTU tables were used for the following analysis. Rarefaction curves, Chao and Shannon indices, Venn diagrams, community barplots at different taxonomic levels, and community heatmap analysis were employed to analyze the microbial community structure of the agarwood samples. Bacterial functional profile was used to compare the gene function at different levels derived from the variational microbial community abundance. The raw sequences of all samples reported in

349 this study have been submitted to the National Center for Biotechnology Information Short Read
350 Archive (accession no. SRP127004) under BioProject PRJNA422987.

351 **Statistical analysis.** The results were reported as means $\pm$ SD. Data were analyzed using IBM SPSS
352 Statistics 20. Statistically significant data were further analyzed using one-way analysis. Significant
353 difference was considered at $p < 0.05$.

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360 **AUTHOR CONTRUBUTIONS**

361 Q.G. Mo completed the experiments via culture-independent methods, interpreted data and developed a
362 manuscript draft. C.Y. Fan and H.Y. Fu completed the experiments via culture-dependent methods. G.
363 Zhou supervised the collection of agarwood samples. Y.W. Wang supervised the whole research and
364 rewrote this manuscript.

365 **CONFLICT OF INTEREST**

366 The authors declare that they have no competing interests.

367 **ETHICAL APPROVAL**

368 This article does not contain any studies with human participants or animals performed by any of the
369 authors.

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375 REFERENCES

- 376 1. Jiao LC, Yin YF, Cheng YM, Jiang XM. 2014. DNA barcoding for identification of the
377 endangered species *Aquilaria sinensis* : comparison of data from heated or aged wood samples.
378 *Holzforschung* 68:487-494. [Http://doi: 10.1515/hf-2013-0129](http://doi: 10.1515/hf-2013-0129)
- 379 2. Wang XH, Gao BW, Liu X, Dong XJ, Zhang ZX, Fan HY, Zhang L, Wang J, Shi SP, Tu PF. 2016.
380 Salinity stress induces the production of 2-(2-phenylethyl) chromones and regulates novel classes
381 of responsive genes involved in signal transduction in *Aquilaria sinensis* calli. *BMC Plant Biol*
382 16:119. [Http://doi: 10.1186/s12870-016-0803-7](http://doi: 10.1186/s12870-016-0803-7)
- 383 3. Wagh VD, Korinek M, Lo I, Hsu YM, Chen SL, Hsu HY, Hwang TL, Wu YC, Chen BH, Cheng
384 YB, Chang FR. 2017. Inflammation modulatory phorbol esters from the seeds of *Aquilaria*
385 *malaccensis*. *J Nat Prod* 80:1421-1427. [Http://doi: 10.1021/acs.jnatprod.6b01096](http://doi: 10.1021/acs.jnatprod.6b01096)
- 386 4. Naef R. 2010. The volatile and semi-volatile constituents of agarwood, the infected heartwood of
387 *Aquilaria* species: a review. *Flavour Frag J* 26:73-87. [Http://doi: 10.1002/ffj.2034](http://doi: 10.1002/ffj.2034)
- 388 5. Dahham SS, Tabana YM, Hassan LEA, Ahamed MBK, Majid ASA, Majid AMSA. 2016. In vitro,
389 antimetastatic activity of agarwood (*Aquilaria crassna*) essential oils against pancreatic cancer
390 cells. *Alex J Med* 52:141-150.
- 391 6. Zhu ZX, Gu YF, Zhao YF, Song YL, Li J, Tu PF, 2016. Gyf-17, a chloride substituted 2-(2-
392 phenethyl)-chromone, suppresses LPS-induced inflammatory mediator production in raw264.7
393 cells by inhibiting STAT1/3 and ERK1/2 signaling pathways. *Int Immunopharmacol* 35:185-192.
394 [Http://doi: 10.1016/j.intimp.2016.03.044](http://doi: 10.1016/j.intimp.2016.03.044)
- 395 7. Gao ZH, Wei JH, Yang Y, Zhang Z, Xiong HY, Zhao WT. 2012. Identification of conserved and
396 novel microRNAs in *Aquilaria sinensis* based on small RNA sequencing and transcriptome
397 sequence data. *Gene* 505:167-175. [Http://doi: 10.1016/j.gene.2012.03.072](http://doi: 10.1016/j.gene.2012.03.072)
- 398 8. Pojanagaroon S, Kaewrak C. 2005. Mechanical methods to stimulate aloes wood formation in
399 *Aquilaria crassna* Pierre ex H.Lec. (Kritsana) trees. *ISHS Acta Hort* 676:161-166.
- 400 9. Liu YY, Chen HQ, Yang Y, Zhang Z, Wei JH, Meng H, Chen WP, Feng JD, Gan BC, Chen XY,
401 Gao ZH, Huang JQ, Chen B, Chen HJ. 2013. Whole-tree agarwood-inducing technique: an
402 efficient novel technique for producing high-quality agarwood in cultivated *Aquilaria sinensis*
403 trees. *Molecules* 18:3086. [Http://doi: 10.3390/molecules18033086](http://doi: 10.3390/molecules18033086)

- 104 10. Zhang Z, Yang Y, Wei JH, Meng H, Sui C, Chen HQ. 2010. Advances in studies on mechanism of
105 agarwood formation in *Aquilaria sinensis* and its hypothesis of agarwood formation induced by
106 defense response. Chin Tradit Herbal Drugs 41:156-159.
- 107 11. Zhang XL, Liu YY, Wei JH, Yang Y, Zhang Z, Huang JQ, Chen HQ, Liu YJ. 2012. Production of
108 high-quality agarwood in *Aquilaria sinensis* trees via whole-tree agarwood-induction technology.
109 Chin Chem Lett 23:727-730. [Http://doi: 10.1016/j.cclet.2012.04.019](http://doi:10.1016/j.cclet.2012.04.019)
- 110 12. Chen XY, Sui C, Liu YY, Yang Y, Liu PW, Zhang Z, Wei JH. 2017. Agarwood formation induced
111 by fermentation liquid of *Lasiodiplodia theobromae*, the dominating fungus in wounded wood of
112 *Aquilaria sinensis*. Curr Microbiol 74:460-468. [Http://doi: 10.1007/s00284-016-1193-7](http://doi:10.1007/s00284-016-1193-7)
- 113 13. Zhang Z, Wei J, Han XM, Liang L, Yang Y, Meng H, Xu YH, Gao ZH. 2014. The sesquiterpene
114 biosynthesis and vessel-occlusion formation in stems of *Aquilaria sinensis* (Lour.) Gilg trees
115 induced by wounding treatments without variation of microbial communities. Int J Mol Sci
116 15:23589-23603. [Http://doi:10.3390/ijms151223589](http://doi:10.3390/ijms151223589)
- 117 14. Tian JJ, Gao XX, Zhang WM, Wang L, Qu LH. 2013. Molecular identification of endophytic
118 fungi from *Aquilaria sinensis* and artificial agarwood induced by pinholes-infusion technique. Afr
119 J Biotechnol 12:3115-3131.
- 120 15. Gao XX, Zhou WP, Zhong ZJ, Liu XJ, Zhang WM, Wu GQ. 2012. Research on the correlation
121 between benzyl acetone and extract content in agarwood. Chin Med Mater 35:919-924.
- 122 16. Zhong ZJ, Fan YF, Lei ZD, Pan QL, Zhou X, Liu DL, Zhang WM, Gao XX. 2016. Determination
123 of agaropiric acid in natural agarwood by HPLC. Lishizhen Med Mater Med Res 27:21-24.
- 124 17. Zhou X, Fan YF, Lei ZD, Pan QL, Zhong ZJ, Liu DL, Zhang WM, Gao XX. 2016. Correlation
125 between agaropiric acid and extract contents in artificial *Aquilariae lignum resinatum*. Chin J Exp
126 Tradit Med Form 22:55-59.
- 127 18. Zou XT. 2015. The study of analysis of changes on the endophytic of *Aquilaria sinensis* in
128 agarwood formation and normal white wood. Dissertation, Guangzhou university of Chinese
129 medicine.[http://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CMFD&dbname=CMFD201502&f](http://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CMFD&dbname=CMFD201502&filename=1015363578.nh&uid)
130 [ilename=1015363578.nh&uid](http://kns.cnki.net/KCMS/detail/detail.aspx?dbcode=CMFD&dbname=CMFD201502&filename=1015363578.nh&uid)
- 131 19. Liang R, Duncan KE, Borgne SL, Davidova I, Yakimov MM, Suflita JM. 2017. Microbial
132 activities in hydrocarbon-laden wastewaters: Impact on diesel fuel stability and the biocorrosion of
133 carbon steel. J Biotechnol 256:68-75. [Http://doi: 10.1016/j.jbiotec.2017.02.021](http://doi:10.1016/j.jbiotec.2017.02.021)

- 434 20. Lee YY, Kim TG, Cho KS. 2015. Effects of proton exchange membrane on the performance and
435 microbial community composition of air-cathode microbial fuel cells. J Biotechnol 211:130-137.
436 [Http://doi: 10.1016/j.jbiotec.2015.07.018](http://doi:10.1016/j.jbiotec.2015.07.018)
- 437 21. Pii Y, Borruso L, Brusetti L, Crecchio C, Cesco S, Mimmo T. 2016. The interaction between iron
438 nutrition, plant species and soil type shapes the rhizosphere microbiome. Plant Physiol Bioch
439 99:39-48. [Http://doi: 10.1016/j.plaphy.2015.12.002](http://doi:10.1016/j.plaphy.2015.12.002)
- 440 22. Hong YW, Liao D, Hu AY, Wang H, Chen JS, Khan S, Su JQ, Li H. 2015. Diversity of
441 endophytic and rhizoplane bacterial communities associated with exotic *Spartina alterniflora* and
442 native mangrove using illumina amplicon sequencing. Can J Microbiol 61:723-733. [Http:// doi: 10.1139/cjm-2015-0079](http://doi:10.1139/cjm-2015-0079)
- 443 23. Xu XH, Zhang Z, Hu SL, Ruan ZP, Jiang JD, Chen C, Shen ZG. 2017. Response of soil bacterial
444 communities to lead and zinc pollution revealed by Illumina MiSeq sequencing investigation.
445 Environ Sci Pollut Res 24:666-675. [Http://doi: 10.1007/s11356-016-7826-3](http://doi:10.1007/s11356-016-7826-3)
- 446 24. Gill AS, Lee A, McGuire KL. 2017. Phylogenetic and functional diversity of total (DNA) and
447 expressed (RNA) bacterial communities in urban green infrastructure bioswale soils. Appl Environ
448 Microbiol 83:e00287-17. [Http://doi:10.1128/AEM.00287-17](http://doi:10.1128/AEM.00287-17)
- 449 25. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, Eisen JA,
450 Sundaresan V. 2015. Structure, variation, and assembly of the root-associated microbiomes of rice.
451 Proc Natl Acad Sci USA 112:E911-E920. [Http://doi: 10.1073/pnas.1414592112](http://doi:10.1073/pnas.1414592112)
- 452 26. Bulgarelli D, Rott M, Schlaeppi K, Bulgarelli D, Rott M, Schlaeppi K, Themaat EVLV,
453 Ahmadinejad N, Assenza F, Rauf P, Huettel B, Reinhardt R, Schmelzer E, Peplies J, Gloeckner
454 FO, Amann R, Eickhorst T, Schulze-Lefert P. 2012. Revealing structure and assembly cues for
455 *Arabidopsis* root-inhabiting bacterial microbiota. Nature 488:91-95.
456 [Http://doi:10.1038/nature11336](http://doi:10.1038/nature11336)
- 457 27. Fiegna F, Velicer GJ. 2005. Exploitative and hierarchical antagonism in a cooperative bacterium.
458 PloS Biol 3:1980-1987. [Http://doi: 0.1371/journal.pbio.0030370](http://doi:10.1371/journal.pbio.0030370)
- 459 28. Sharma RR, Dinesh S, Rajbir S. 2009. Biological control of postharvest diseases of fruits and
460 vegetables by microbial antagonists: a review. Biol Control 50:205-221. [Http://doi: 10.1016/j.biocontrol.2009.05.001](http://doi:10.1016/j.biocontrol.2009.05.001)

29. Mohamed R, Jong PL, Zali MS. 2010. Fungal diversity in wounded stems of *Aquilaria malaccensis*. Fungal Divers 43:67-74. [Http://doi: 10.1007/s13225-010-0039-z](http://doi.org/10.1007/s13225-010-0039-z)
30. Premalatha K, Kalra A. 2013. Molecular phylogenetic identification of endophytic fungi isolated from resinous and healthy wood of *Aquilaria malaccensis*, a red listed and highly exploited medicinal tree. Fungal Ecol 6:205-211. [Http://doi: 10.1016/j.funeco.2013.01.005](http://doi.org/10.1016/j.funeco.2013.01.005)
31. Langille MGI, Zaneveldm J, Caporasom JG, Mcdonald D, Dan K, Reyes JA, Clemente J, Clemente JC, Burkepile DE, Thurber RLV, Rob K, Beiko RG, Huttenhower C. 2013. Predictive functional profiling of microbial communities using 16s rRNA marker gene sequences. Nat Biotechnol 31: 814-823. [Http://doi: 10.1038/nbt.2676](http://doi.org/10.1038/nbt.2676)
32. Chiriac CM, Szekeres E, Rudi K, Baricz A, Hegedus A, Dragoş N, Coman C. 2017. Differences in temperature and water chemistry shape distinct diversity patterns in thermophilic microbial communities. Appl Environ Microbiol 83:e01363-17. [Http://doi:10.1128/AEM.01363-17](http://doi.org/10.1128/AEM.01363-17)
33. Ye W, Wu HQ, He X, Wang L, Zhang WM, Li HH, Fan YF, Tian GH, Liu TM, Gao XX. 2016. Transcriptome sequencing of chemically induced *Aquilaria sinensis* to identify genes related to agarwood formation. PloS One 11:e0155505. [Http://doi: 10.1371/journal.pone.0155505](http://doi.org/10.1371/journal.pone.0155505)
34. Ju F, Zhang T. 2015. 16S rRNA gene high-throughput sequencing data mining of microbial diversity and interactions. Appl Microbiol Biotechnol 99:4119-4129. [Http://doi: 10.1007/s00253-015-6536-y](http://doi.org/10.1007/s00253-015-6536-y)
35. Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340-1351. [Http://doi: 10.1038/ismej.2010.58](http://doi.org/10.1038/ismej.2010.58)
36. Jayawardena RS, Purahong W, Zhang W, Wubet T, Li X, Liu M, Zhao WS, Hyde KD, Liu JH, Yan JY. 2018. Biodiversity of fungi on *Vitis vinifera* L. revealed by traditional and high-resolution culture-independent approaches. Fungal Divers 1-84. [Http://doi.org/10.1007/s13225-018-0398-4](http://doi.org/10.1007/s13225-018-0398-4)
37. Kanagawa T. 2003. Bias and artifacts in multitemplate polymerase chain reaction (PCR). J Biosci Bioengin 96:317-323. [Http://doi.org/10.1016/S1389-1723\(03\)90130-7](http://doi.org/10.1016/S1389-1723(03)90130-7)
38. Dissanayake AJ, Purahong W, Wubet T, Hyde KD, Zhang W, Xu HY, Zhang GJ, Fu CY, Liu M, Xiang QK, Li XH, Yan JY. 2018. Direct comparison of culture-dependent and culture-independent molecular approaches reveal the diversity of fungal endophytic communities in stems of grapevine (*Vitis vinifera*). Fungal Divers 90:85-107. [Http://doi: 10.1007/s13225-018-0399-3](http://doi.org/10.1007/s13225-018-0399-3)

39. Ye W, He X, Wu HQ, Wang L, Zhang WM, Fan YF, Li HH, Liu TM, Gao XX. 2018. Identification and characterization of a novel sesquiterpene synthase from *Aquilaria sinensis*: an important gene for agarwood formation. *Int J Biol Macromol* 108:884-892. [Http://doi: 10.1016/j.ijbiomac.2017.10.183](http://doi:10.1016/j.ijbiomac.2017.10.183)
40. Xu YH, Zheng Z, Wang MX, Wei JH, Chen HJ, Gao ZH, Sui H, Luo HM, Zhang XL, Yang Y, Meng H, Li WL. 2013. Identification of genes related to agarwood formation: transcriptome analysis of healthy and wounded tissues of *Aquilaria sinensis*. *BMC Genomics* 14:227. [Http://doi: 10.1186/1471-2164-14-227](http://doi:10.1186/1471-2164-14-227)
41. Siah CH, Namasivayam P, Mohamed R. 2016. Transcriptome reveals senescing callus tissue of *Aquilaria malaccensis*, an endangered tropical tree, triggers similar response as wounding with respect to terpenoid biosynthesis. *Tree Genet Genomes* 12:33-42. [Http://doi:10.1007/s11295-016-0993-z](http://doi:10.1007/s11295-016-0993-z)
42. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. 2003. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *P Natl Acad Sci* 100:1541-1546. [Http://doi: 10.1073/pnas.0337542100](http://doi:10.1073/pnas.0337542100)
43. Meguro A, Tomita T, Nishiyama M, Kuzuyama T. 2013. Identification and characterization of bacterial diterpene cyclases that synthesize the membrane skeleton. *Chem Bio Chem* 14(3):316-321. [Http://doi: 10.1002/cbic.201200651](http://doi:10.1002/cbic.201200651)
44. Salomon MV, Bottini R, de Souza Filho GA, Cohen AC, Moreno D, Gil M, Piccoli P. 2014. Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in in vitro cultured grapevine. *Physiol Plantarum* 151:359-374. [Http://doi:10.1111/ppl.12117](http://doi:10.1111/ppl.12117)
45. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, del Rio TG, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* 488:86-90. [Http://doi: 10.1038/nature11237](http://doi:10.1038/nature11237)
46. Bulgarelli D, Garrido-Oter RC, Münch P, Weiman A, Droge J, Pan YC, McHardy A, Schulze-Lefert P. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* 17:392-403. [Http://doi: 10.1016/j.chom.2015.01.011](http://doi:10.1016/j.chom.2015.01.011)

522 47. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña
523 AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone
524 CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA,
525 Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-
526 throughput community sequencing data. *Nat Methods* 7:335-336. [Http://doi:10.1038/nmeth.f.303](http://doi:10.1038/nmeth.f.303)
527 48. Xiao X, Fan M, Wang E, Chen W, Wei G. 2017. Interactions of plant growth-promoting
528 rhizobacteria and soil factors in two leguminous plants. *Appl Microbiol Biotechnol* 101:8485-8497.
529 [Http://doi: 10.1007/s00253-017-8550-8](http://doi:10.1007/s00253-017-8550-8)
530 49. Berasategui A, Axelsson K, Nordlander G, Schmidt A, Borg-Karlson A, Gershenzon J, Terenius O,
531 Kaltenpoth K. 2016. The gut microbiota of the pine weevil is similar across Europe and resembles
532 that of other conifer-feeding beetles. *Mol Ecol* 25:4014-4031. [Http://doi: 10.1111/mec.13702](http://doi:10.1111/mec.13702)
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552 **Figure captions**

553 **FIG 1** Community richness and community diversity indices at a 97% identity threshold of the natural
554 agarwood (NA) and agar-wit agarwood (Agar-Wit). a and c are the Chao indices of bacterial and fungal
555 communities, respectively. b and d are the Shannon indices of bacterial and fungal communities,
556 respectively. The values are presented as means $\pm$ SD.

557 **FIG 2** Venn diagrams depicting the shared or unique OTUs for natural agarwood (NA) and agar-wit
558 agarwood (Agar-Wit). The OTU abundance with $< 1\%$ are combined and defined as others. a and b are
559 bacterial and fungal OTUs, respectively. c and d are the main shared OTUs in bacterial and fungal
560 types, respectively.

561 **FIG 3** Bacterial and fungal compositions of natural agarwood (NA) and agar-wit agarwood (Agar-Wit)
562 on the phyla (a, b) and order (c, d) levels. a and c are the bacterial communities, and b and d are the
563 fungal communities. Others include the few sequences with $< 1\%$ relative abundance.

564 **FIG 4** Heatmap of the bacterial (a) and fungal (b) distributions of the top 50 most abundant genera
565 presented in the natural agarwood (NA) and agar-wit agarwood (Agar-Wit). The relative abundances of
566 bacterial and fungal genera are indicated by color intensity. In the current study, the total fungal
567 community types of these two samples were 25.

568 **FIG 5** Taxon-level analysis of individual genera or orders altered between the natural agarwood (NA)
569 and agar-wit agarwood (Agar-Wit) by Fisher's exact test. a: Bacterial communities at the genus level; b:
570 fungal communities at the order level. The right graph indicates the altered percentage of species
571 abundance in the confidence interval. $**0.001 < p < 0.01$, $***p < 0.001$.

572 **FIG 6** Abundance and functional predictions of bacterial communities of the natural agarwood (NA)
573 and agar-wit agarwood (Agar-Wit). a: COG functional predictions of OTUs. b and c: KEGG functional
574 predictions of OTUs by using the PICRUSt tool in category levels 1 and level 2, respectively.

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580 **TABLE 1** The final taxon of all fungal OTUs detected from high-throughput sequencing.

Final Taxon	OTU	Abundance (%)	
		NA	Agar-Wit
<i>Agaricomycetes</i>	OTU31	0.095	8.06
<i>Arthrobotrys oligospora</i>	OTU33	0.855	0.839
<i>Ascomycota</i>	OTU2	10.669	0.008
<i>Ascomycota</i>	OTU19	0.576	0.004
<i>Ascomycota</i>	OTU14	0.052	0
<i>Aspergillus</i>	OTU26	1.217	3.655
<i>Basidiomycota</i>	OTU37	0	0.114
<i>Botryosphaeriaceae</i>	OTU30	5.82	2.18
<i>Candida parapsilosis</i>	OTU38	0	0.004
<i>Capnodiales</i>	OTU4	12.303	0.179
<i>Coniochaetaceae</i>	OTU29	7.235	46.194
<i>Corticaceae</i>	OTU20	13.399	0
<i>Dothideomycetes sp. CRI7</i>	OTU13	0.133	0
<i>Flammispora bioteca</i>	OTU24	1.977	0
Fungi	OTU11	0.112	0
<i>Graphis scripta</i>	OTU5	0.052	0
<i>Herpotrichiellaceae</i>	OTU34	6.203	2.995
<i>Hydnodontaceae</i>	OTU18	2.889	0
<i>Hypocreales</i>	OTU8	0.176	0
<i>Kurtzmanomyces insolitus</i>	OTU12	2.489	0.11
<i>Lecanoromycetes</i>	OTU3	3.791	0.008
<i>Rhizoctonia solani</i>	OTU22	2.093	0
<i>Sordariales</i>	OTU16	7.677	0.509
<i>Sordariales</i>	OTU27	0.047	0.098
<i>Sordariomycetes</i>	OTU36	3.417	25.446
<i>Sordariomycetes</i>	OTU28	15.063	9.518
<i>Sordariomycetes</i>	OTU32	1.221	0.016
<i>Sordariomycetes</i>	OTU9	0.06	0

<i>Tremellaceae</i>	OTU15	0.064	0
<i>Tremellales</i>	OTU10	0.314	0.061

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 NA: natural agarwood; Agar-Wit: agar-wit agarwood.

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