Soil microbial diversity and functional capacity associated with the production of edible mushroom *Stropharia rugosoannulata* in croplands

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In recent years, a rare edible mushroom *Stropharia rugosoannulata* has become popular. *S. rugosoannulata* has the characteristics of easy cultivation, low cost, high output value, and low labor requirement, making its economic benefits significantly superior to those of other planting industries . Accumulating research demonstrates that cultivating edible fungus is advantageous for farming soil. The present experiment used idle croplands in winter for *S. rugosoannulata* cultivation. We explored the effects of *S. rugosoannulata* cultivation on soil properties and soil microbial community structure in paddy and dry fields, respectively. We cultivated *S. rugosoannulata* in the fields after planting chili and rice, respectively. The results showed that Chili-*S. rugosoannulata* and Rice-*S. rugosoannulata* planting patterns increased the yield, quality and amino acid content of *S. rugosoannulata*. By analyzing the soil properties, we found that the Chili-*S. rugosoannulata* and Rice-*S. rugosoannulata* cropping patterns increased the total nitrogen, available phosphorus, soil organic carbon, and available potassium content of the soil. We used 16s amplicons for bacteria and internal transcribed spacer (ITS) region for fungi to analyze the microbial communities in rhizosphere soils. Notably, *S. rugosoannulata* cultivation significantly increased the abundance of beneficial microorganisms such as *Chloroflexi*, *Cladosporium* and *Mortierella* and reduce the abundance of *Botryotrichumin* and *Archaeorhizomyces*. We consider *S. rugosoannulata* cultivation in cropland can improve soil properties, regulate the community structure of soil microorganisms, increase the expression abundance of beneficial organisms and ultimately improve the *S. rugosoannulata* yield and lay a good foundation for a new round of crops after this edible mushroom cultivation.

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15 Abstract

16 In recent years, a rare edible mushroom *Stropharia rugosoannulata* has become popular. *S.*
17 *rugosoannulata* has the characteristics of easy cultivation, low cost, high output value, and low
18 labor requirement, making its economic benefits significantly superior to those of other planting
19 industries. Accumulating research demonstrates that cultivating edible fungus is advantageous
20 for farming soil. The present experiment used idle croplands in winter for *S. rugosoannulata*
21 cultivation. We explored the effects of *S. rugosoannulata* cultivation on soil properties and soil
22 microbial community structure in paddy and dry fields, respectively. We cultivated *S.*
23 *rugosoannulata* in the fields after planting chili and rice, respectively. The results showed that
24 Chili-*S. rugosoannulata* and Rice-*S. rugosoannulata* planting patterns increased the yield,
25 quality and amino acid content of *S. rugosoannulata*. By analyzing the soil properties, we found
26 that the Chili-*S. rugosoannulata* and Rice-*S. rugosoannulata* cropping patterns increased the
27 total nitrogen, available phosphorus, soil organic carbon, and available potassium content of the
28 soil. We used 16s amplicons for bacteria and internal transcribed spacer (ITS) region for fungi to
29 analyze the microbial communities in rhizosphere soils. Notably, *S. rugosoannulata* cultivation
30 significantly increased the abundance of beneficial microorganisms such as *Chloroflexi*,
31 *Cladosporium* and *Mortierella* and reduce the abundance of *Botryotrichumin* and
32 *Archaeorhizomyces*. We consider *S. rugosoannulata* cultivation in cropland can improve soil
33 properties, regulate the community structure of soil microorganisms, increase the expression
34 abundance of beneficial organisms and ultimately improve the *S. rugosoannulata* yield and lay a
35 good foundation for a new round of crops after this edible mushroom cultivation.

36 Introduction

37 The edible fungi industry is an important industry in the global planting industry, and most
38 edible fungi extracts have biological activities such as antioxidants[1, 2]. *S. rugosoannulata* is a
39 rare edible mushroom originally discovered by Professor Murrill in the United States [3].

40   Because of its high economic and ecological value, *S. rugosoannulata* was introduced to China
41   as early as the 1980s and has been widely planted [3]. Experimental pharmacological studies
42   have shown that *S. rugosoannulata* has various biological activities such as antitumor,
43   antibacterial, antioxidant, and immunomodulatory [4, 5]. In recent years, the demand for *S.*
44   *rugosoannulata* has been increasing due to its delicious and pharmacological properties, so it is
45   urgent to increase its production [6].
46   Crop rotation appears to be an effective method for boosting *S. rugosoannulata* output [7]. This
47   is due to long-term inappropriate use of the land, such as diffuse irrigation, excessive use of
48   fertilizers, pesticides, etc., can lead to damage to the soil structure, imbalance of nutrient ratios,
49   degradation, and acidification [8]. Rotation can increase the soil organic carbon and total
50   nitrogen content, improve soil fertility, and boost crop output [9, 10]. The mycelium produced by
51   growing edible mushrooms contains various trace elements [11]. The residual mycelium is rich
52   in crude protein, crude fat, calcium, phosphorus, and other organic substances [12]. Some
53   bioactive substances secreted by the mycelium in the production process can decompose
54   complex organic matter, inhibit some soil-borne diseases and promote plant growth [13]. The
55   texture of the mushroom residue is loose and has a solid ability to absorb water, which can be
56   further decomposed into humus with good air and water storage capacity and can effectively
57   improve the soil [14]. This indicates that mushroom residue can well regulate the soil properties
58   of agricultural land and enhance soil fertility. In addition, crop cultivation in cropland also
59   contributes to mushroom culture [15]. That is because the rotation of different crops on the same
60   soil can significantly improve the physical and chemical properties of the soil, regulate soil
61   fertility, and ultimately achieve increased production and income [16].
62   Chili and rice are two important economic crops in China's Hunan Province, and both have a
63   positive impact on soil quality. Therefore, it seems to be a good choice to cultivate *S.*
64   *rugosoannulata* in winter on unused acreage. Studies have indicated that using chili as a crop
65   rotation can greatly enhance soil nitrogen content and minerals after planting, as well as increase
66   carbon fixation in soil [17]. Mi-Seon Hahm et al. discovered that using chili as a crop rotation
67   boosted the expression abundance of *Chloroflexi* in soil while decreasing salt accumulation [18].
68   In addition to regulating soil microorganisms, chili inhibits the development and proliferation of
69   *Meloidogyne* spp., according to research conducted by Bafokuzara [19]. Some experiments have
70   found that the implementation of paddy upland rotations increases the stability of soil physical
71   properties, improves the bulk density of soil, reduces damage to soil structure, and increases soil
72   water stable aggregate[20]. On the other hand, unlike dryland soils alone, soils in paddy upland
73   rotations change the chemical form of the nutrients in them due to alternating wet and dry,
74   anaerobic and aerobic conditions [21]. Crop rotation can effectively improve soil utilization,
75   improve soil structure through the roots of different crops, reduce soil consolidation and
76   degradation, and increase soil nutrient content[22].
77   Currently, the majority of research use horticultural crops in rotation with chili or rice. Few
78   studies have investigated the influence of edible fungus as rotation crops on soil characteristics.
79   In this experiment, *S. rugosoannulata* was used for the first time to rotate with chili and rice.

80   Additionally, *S. rugosoannulata* was cultivated by maximizing the use of idle farmland during
81   the winter, and it was investigated whether the cultivation process as a whole could regulate soil
82   properties and soil microorganisms. This study will contribute to the commercial production of
83   *S. rugosoannulata* and enhance the utilization of vacant agricultural land in winter.

84   Materials & Methods

85   Experimental design

86   Three experimental plots of the same size (2 hectare) in the experimental field of the Hunan
87   Institute of Microbiology (longitude: 112.98, Latitude: 28.12) were selected. These three plots
88   have the same size and adjacent positions. The experiment began in April 2020. The chili variety
89   used in the experiment was (*Capsicum annuum L.*), 'SJ11-3'. The rice variety used in the
90   experiment was "Zhongjiazao 17". Cultivation of *S. rugosoannulata* in vacant fields (NC),
91   cultivated *S. rugosoannulata* after harvested chili (Chili-*S. rugosoannulata*) and rice (Rice-*S.*
92   *rugosoannulata*) crops. In April 2020, chili was planted in the experimental field of the Chili-*S.*
93   *rugosoannulata* group. Chili harvested in October 2020. In June, rice was planted in the
94   experimental field of the Rice-*S. rugosoannulata* group. Rice harvested in September 2020. In
95   mid-November 2020, the ground of all test fields was leveled and all crop residues were removed
96   and not plowed into the soil. The quicklime was used to disinfect and kill insects on the fields.
97   Soak straw, corncob, and chaff in 3 % lime water for two days, then mix them in an 8:1:1 ratio
98   and distribute them equally with a thickness of 30 cm on all experimental fields. Paving it with
99   the *S. rugosoannulata* spawn (300 kg/mu) and covering it with approximately 2-3 cm of soil.
100 After covering the soil, cover it with a layer of rice straw (the covering layer is not visible, the
101 thickness is 3-4cm). Three to four months were required for the development of the fungi to
102 fruiting bodies. The root soil from the three experimental fields was sampled the same year in
103 mid –November, next year in January and March to see the effect of fungal growth on the soil
104 quality. We used the five-point sampling method to produce a composite sample by combining
105 five independent soil cores from around and within the croplands[23]. Six composite samples
106 were taken from each experimental field. Fresh samples were sealed in plastic bags and stored in
107 a -80° freezer.

108 Determination of amino acid content in *S. rugosoannulata*

109 After crushing *S. rugosoannulata* through a 50-mesh sieve (the aperture is 0.3 mm), add 2 ml of
110 concentrated hydrochloric acid, mix 2 to 3 drops of phenol, and evacuate, heat melt the glass
111 hydrolysis tube while keeping the hydrolysis tube in a high vacuum state, and complete the
112 sealing. Place the hydrolysis tube in an oven at 110°C. After hydrolyzing for 24 hours, take it out
113 and cool it to room temperature. Transfer the hydrolyzed sample to a 25ml volumetric flask.
114 Wash the hydrolysis tube repeatedly with pure water and fill the volume up to the mark. After
115 the constant volume of the sample is passed through a 0.22μm microporous membrane, put it
116 into the sample vial to be tested. Analysis and identification of amino acids in biological tissues
117 utilizing a High-performance liquid chromatograph (Model 1100, Agilent, USA). The
118 chromatographic conditions are: chromatographic column: Zorbax Eclipse AAA C18 column (75
119 mm × 4.6 mm, 3.5 μm, Agilent, USA); mobile phase: A is 40 mmol/L NaH_2PO_4 solution, pH

120 7.8; B is acetonitrile-methanol-water (volume ratio of 4.5:4.5:1). Mobile phase gradient elution
121 program: 0.0 - 1.0 min (B: 0%), 1.0 - 9.8 min (B: 0%-57%), 9.8-10.0 min (B: 57% ~100%), 10.0
122 - 12.0 min (B: 100%), 12.0 - 12.5 min (B: 100% ~ 0%), 12.5 - 14.0 min (B: 0%); flow rate: 2
123 mL/min; column temperature: 40°C; injection volume: 18 μl.

124 **Determination of soil total nitrogen**
125 1.00 g air-dried soil sample with 100-mesh sieve (the aperture is 0.15 mm) was weighed and
126 carefully placed in the Kjeldahl flask. A small amount of distilled water was added for wetting
127 and washing. Then, 2 g of the prepared mixed catalyst was added, and 5 ml of concentrated
128 sulfuric acid was added to cover the bottle cap with a small funnel. Place the prepared bottle on
129 the digester at 375 °C for 1 hour. The end of cooking is a clear light blue mixture of the cooking
130 furnace. After digestion, the Kai-type bottle was taken out, standing for cooling, shaking in the
131 middle for several times to prevent precipitation, and the small funnel was taken down. The
132 small funnel was washed with distilled water to make all the solution in the small funnel enter
133 the Kai-type bottle. The supernatant was taken and diluted to adjust the pH value. The total
134 nitrogen content in the solution was measured by a flow analyzer (AA3Auto Analyzer 3
135 Continuous-Flow Analysis-3, SEAL, Germany), and then converted into soil total nitrogen
136 content[24]

137 **Determination of soil available phosphorus**
138 Weigh 2.5 g air-dried soil sample which has passed through a 20-mesh sieve (the aperture is 0.85
139 mm), add 50 ml $NaHCO_3$ (0.5 mol·L-1) solution and phosphorus-free activated carbon, shake for
140 30 minutes, and filter. Remove the filtrate and add 2-6 dinitrophenol color reagent, adjust the
141 solution to slightly yellow with diluted acid, add Mo-Sb antimony reagent and read the
142 absorbance value at 720 nm wavelength[25].

143 **Determination of soil organic carbon**
144 Weigh a soil sample that has been quantitatively passed through an 80-mesh sieve (the aperture
145 is 0.18 mm) placed in a dry hard test tube, with the transfer tube to accurately add 0.8000 mol/L
146 $(1/6K_2CrO_7)$ standard solution 5 m L, accurately add concentrated sulfuric acid 5 ml fully shake.
147 The test tube was placed in a wire cage, and then the wire cage was placed in an oil bath to heat.
148 After being placed, the temperature should be controlled at 170-180 °C. When the liquid in the
149 test tube boils and bubbles occur, it starts to count, boils for 5 min, removes the test tube, slightly
150 cools, and wipes out the external oil of the test tube. Take out the cooling. After cooling, all the
151 contents in the test tube were carefully transferred into 250 ml triangle bottle, so that the volume
152 of the contents in the triangle bottle was 60-70 ml. Keep sulfuric acid concentration of 1~1.5 mol
153 / l, the solution color should be orange or light yellow. Then add o-norphine indicator 3~4 drops,
154 titrate with 0.2 mol/l standard ferrous sulfate $(FeSO_4)$ solution, the solution from yellow through
155 green, light green to brown is the end point[26].

156 **Determination of soil moisture**
157 Weigh a 5 g soil sample, put it in an oven at 105 °C, and bake it until the weighing remains
158 constant before and after twice. Loss of moisture after drying is the soil moisture content.

159 **Determination of soil available potassium**

160 Weigh a 5 g air-dried soil sample which has passed through a 20-mesh sieve (the aperture is 0.85
161 mm), add 50 ml NH_4OAc (1 mol·L^{-1}) solution, shake for 30 minutes, filter, and take the filtrate
162 for measurement on a flame photometer[27].

163 **Soil DNA extraction and library preparation**

164 Use EZNA® soil DNA kit (Omega Bio-tek, Norcross, GA, USA) to extract DNA from soil
165 samples. The primer pair 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-
166 GGACTACHVGGGTWTCTAAT-3') was used to amplify the bacterial 16S V3+V4 region of
167 extract DNA. The fungal ITS2 region of extract DNA was amplified using primer pair ITS2F (5'-
168 GCATCGATGAAGAACGCAGC-3') and ITS2R (5'-TCCTCCGCTTATTGATATGC-3'). PCR
169 reaction conditions were 25 cycles of 95 °C 5min, 95 °C 30 sec, 50 °C 30 sec, 72 °C 40 sec, and
170 finally 72 °C for 7 min, and stored at 4 °C. The PCR products obtained were quantified by
171 electrophoresis (ImageJ software) and mixed in a mass ratio of 1:1. Column purification was
172 performed using OMEGA DNA purification columns (bio-tek, Doraville, GA, USA). Finally,
173 use 1.8% agarose gel, electrophoresis at 120 V for 40min, and cut the gel to recover the target
174 fragment.

175 **Illumina sequencing and microbial diversity analysis**

176 The samples were sequenced on the Illumina MiSeq platform, the original data were spliced
177 (FLASH, version 1.2.11)[28], using Trimmomatic (version 0.33) to filter the quality of the raw
178 data[29], then use Cutadapt (version 1.9.1) to identify and remove primer sequences, and then
179 use USEARCH (version 10) to splice paired-end reads and remove chimeras (UCHIME, version
180 8.1) finally obtained high-quality sequences for subsequent analysis[30]. Sequences with a
181 similarity of more than 97% were clustered as an OTU (USEARCH, version 10.0), and 0.005%
182 of all sequences sequenced were used as a threshold to filter OTUs[31]. Bacteria were annotated
183 using the Silva database (Release128, http://www.arb-silva.de), and fungi were annotated using
184 Unite (Release 7.2, http://unite.ut.ee/index.php). Species annotation was performed using RDP
185 Classifier with a confidence threshold of 0.8 (version 2.2,
186 http://sourceforge.net/projects/rdpclassifier/). Using the Mothur version (v.1.30) (HTTP://
187 www.mothur.org/) for Alpha diversity index analysis. All sample sequences were uploaded to
188 the NCBI database, with sample numbers ranging from SRX13373992 to SRX13374011.

189 **Correlation and predictive analysis of microbial function**

190 Correlation analysis and mapping of soil quality and soil microorganisms using the R language
191 vegan (v2.3) package. RDA (Redundancy analysis) is a sorting method developed based on
192 correspondence analysis; RDA analysis is based on a linear model, mainly used to reflect the
193 relationship between flora or samples and environmental factors. The size of the first axis of
194 Lengths of a gradient in the analysis result should be less than4.0. PICRUSt2 (Phylogenetic
195 Investigation of Communities by Reconstruction of Unobserved States 2) software was used to
196 predict the abundance of marker functional gene sequences in samples. PICRUSt2 is a
197 computational method that utilizes marker gene data and a reference genome database to predict
198 the functional composition of environmental microbes, based on IMG microbial genome data to
199 predict microbial communities' functional potential during phylogeny through phylogeny

200 phylogenetic-functional correlations. Based on the software's reference genome data, both 16S
201 rRNA sequences and ITS sequences can be used for functional prediction [32].

202 **Determination of *S. rugosoannulata* quality**

203 Take the fruiting body of *S. rugosoannulata*, measure the diameter and length of its stipe, and
204 determine whether it is hollow. 5 kg of fruiting bodies were randomly selected in each
205 experimental field to evaluate the quality of *S. rugosoannulata*.

206 **Data Analysis**

207 All data in this study were analyzed using SPSS 22.0, expressed as mean ± standard deviation of
208 the mean (SD). The difference between the means of the groups was analyzed using a one-way
209 ANOVA pair and evaluated using Tukey multiple comparisons. $p < 0.05$ was regarded as a
210 significant difference.

211 # Results

212 **Quality and yield of *S. rugosoannulata* in different croplands**

213 First, we determined the quality of *S. rugosoannulata*. As shown in Figure 1, the mushroom stipe
214 of *S. rugosoannulata* grown in the NC group was elongated and hollow (Figure 1a). However,
215 compared with the NC group, the stipe of *S. rugosoannulata* grown in rotation with chili and rice
216 was significantly thicker (Fig. 1b, c). Among them, *S. rugosoannulata* which was rotated with
217 rice, has the thickest mushroom stipe, the largest volume, and the best quality. The yield of *S.*
218 *rugosoannulata* in different croplands was shown in Figure 1d. Compared with NC, the yield of
219 Chili- *S. rugosoannulata* and Rice-*S. rugosoannulata* was significantly higher ($p < 0.05$). Among
220 them, *S. rugosoannulata*, which was rotated with rice, has the highest yield. It reached about 240
221 kg/hm^2.

222 **Amino acid content of *S. rugosoannulata* in different croplands**

223 Table 1 shows the analysis of amino acid content in *S. rugosoannulata*. For the *S.*
224 *rugosoannulata* in Chili-*S. rugosoannulata*, there was a substantial increase in the level of Thr,
225 Ser, Glu, Gly, Ala, Val, Ile, Leu, Tyr, His, Lys, and Pro ($p < 0.05$) relative to the control *S.*
226 *rugosoannulata*. Meanwhile, for the *S. rugosoannulata* in Rice-*S. rugosoannulata*, the levels of
227 16 types of amino acids increased substantially ($p < 0.05$) in comparison to the control *S.*
228 *rugosoannulata*

229 **Soil properties of different croplands**

230 We found that compared with the NC control group, the Chili-*S. rugosoannulata* and Rice-*S.*
231 *rugosoannulata* rotations significantly improved the total nitrogen (Figure 2a), available
232 phosphorus (Figure 2b), soil organic carbon (Figure 2c) and available potassium (Figure 2d)
233 content in the soil before, during and after cultivating *S. rugosoannulata* ($p < 0.05$). Among
234 them, the soil of *S. rugosoannulata* and rice rotation has the highest content of total nitrogen,
235 available phosphorus, soil organic carbon, and available potassium. Compared with the NC
236 control group, the Chili- *S. rugosoannulata* and Rice-*S. rugosoannulata* rotations significantly
237 improved the soil moisture before and after cultivating *S. rugosoannulata* and it is the highest in
238 group Rice-*S. rugosoannulata* ($p < 0.05$). However, during the cultivating of *S. rugosoannulata*,

239   there was no significant difference in soil moisture among the three groups (Figure 2e) (p >
240   0.05).

241   **The soil rhizosphere microbial community diversity in different croplands**
242   After cultivating *S. rugosoannulata*, Chili- *S. rugosoannulata*, and Rice- *S. rugosoannulata*, two
243   soil rhizosphere microorganisms ACE index, Chao index, Shannon index, and Simpson index
244   were significantly higher than the NC group ($p < 0.05$) (Figure 3a-d).

245   **The structure of soil rhizosphere microbial community in different croplands**
246   At the phylum level, after cultivating *S. rugosoannulata*, *Proteobacteria*, *Acidobacteria*,
247   *Actinobacteria*, and *Chloroflexi* were the main microorganisms in the three groups, accounting
248   for more than 70% of the total abundance (Figure 4a). Compared with the NC group, the
249   abundance of *Proteobacteria*, *Bacteroidetes*, and *Actinobacteriain* in the Chili- *S.
250   rugosoannulata* group after cultivating *S. rugosoannulata* significantly decreased, while the
251   abundance of *Chloroflexi* flora increased significantly ($p < 0.05$). Compared with the NC group,
252   the abundance of *Bacteroidetes* and *Actinobacteriain* in the Rice- *S. rugosoannulata* group after
253   cultivating *S. rugosoannulata* significantly decreased, while the abundance of *Chloroflexi*
254   increased significantly ($p < 0.05$) (Figure 4b-e).
255   At the genus level, the bacterial community composition in the rhizosphere after cultivating *S.
256   rugosoannulata* is shown in Figure 4 F. After cultivating *S. rugosoannulata*, compared with the
257   NC group, the abundance of *Sphingomonas and Burkholderia-Caballeronia-Paraburkholderia*
258   and *Granulicella* in the Chili- *S. rugosoannulata* group was significantly reduced, while the
259   abundance of *Candidatus Solibacter* was significantly increased ($p < 0.05$). Rice- *S.
260   rugosoannulata* group significantly increased the abundance of *Sphingomonas, C. Solibacter* and
261   *Gemmatimonas* ($p < 0.05$) (Figure 4g-j).

262   **The structure of soil rhizosphere fungal community in different croplands**
263   At the phylum level, after cultivating *S. rugosoannulata*, *Basidiomycota*, *Ascomycota*, and
264   *Mortierellomycota* were the main fungi in the three groups, accounting for more than 90% of the
265   total abundance (Figure 5a). Compared with the NC group, the abundance of *Ascomycota* in the
266   Chili- *S. rugosoannulata* group after cultivating *S. rugosoannulata* significantly decreased, while
267   the abundance of *Basidiomycota*, and *Mortierellomycota* increased significantly ($p < 0.05$).
268   Compared with the NC group, the abundance of *Chytridiomycota* in the Chili- *S. rugosoannulata*
269   group after cultivating *S. rugosoannulata* significantly decreased ($p < 0.05$). Compared with the
270   NC group, the abundance of *Ascomycota* in the Rice- *S. rugosoannulata* group after cultivating
271   *S. rugosoannulata* significantly decreased, while the abundance of *Chytridiomycota and
272   Basidiomycota* increased significantly ($p < 0.05$) (Figure 5b-e).
273   At the genus level, the fungal community composition in the rhizosphere after cultivating *S.
274   rugosoannulata* is shown in Figure 5f. After cultivating *S. rugosoannulata*, compared with the
275   NC group, the abundance of *Stropharia and Myrmecridium* in the Chili- *S. rugosoannulata*
276   group was significantly increased, while the abundance of *Chaetosphaeria* and *Scytalidium* was
277   significantly reduced ($p < 0.05$). Rice- *S. rugosoannulata* group significantly increased the

278   abundance of *Stropharia,* but significantly decreased the abundance of *Chaetosphaeria* and
279   *Scytalidium* ($p < 0.05$) (Figure 5g-j).
280   **Function prediction of bacteria and fungi produced in different croplands**
281   We found that soil organic carbon and available potassium had the greatest impact on the entire
282   rotation process of cultivating *S. rugosoannulata* (Figure 6a). We conducted a Pearson
283   correlation analysis on the soil rhizosphere microorganisms at the phylum level and the amino
284   acid content in *S. rugosoannulata*. We found that *Chloroflexi*, *Nitrospirae*, *Acidobacteria* and
285   *Verrucomicrobia* are positively correlated with most amino acid content, while Bacteroidetes,
286   Firmicutes, Actinobacteria and Proteobacteria are negatively correlated with most amino acid
287   content (Figure 6c). We used soil properties as environmental factors, and performed RDA
288   analysis with soil rhizosphere fungi, and found that available phosphorus and available
289   potassium had the greatest impact on the entire rotation process of cultivating *S. rugosoannulata*
290   (Figure 6b). We conducted a Pearson correlation analysis on the soil rhizosphere fungi at the
291   phylum level and the amino acid content in *S. rugosoannulata*. We found that *Rozellomycota*,
292   Chytridiomycota and *Mortierellomycota* are positively correlated with most amino acid content,
293   while *Ascomycota* are negatively correlated with most amino acid content (Figure 6d).
294   Finally, we found that compared with the NC group, the microbes in the Chili-*S. rugosoannulata*
295   group (Figure 7a) and the Rice-*S. rugosoannulata* group (Figure 7b) were two-component
296   system, Microbial metabolism in diverse environments, Quorum Sensing and ABC transporters
297   related functional genes are significantly enriched. We conducted a predictive analysis of the
298   FUNGuild between different groups and found that compared with the NC group, the fungi in
299   the Chili-*S. rugosoannulata* group (Figure 7c) and the Rice-*S. rugosoannulata* group (Figure 7d)
300   were Plant Parasite, Dung Saprotroph and Soil Saprotroph related functional genes are
301   significantly enriched.
302
303   **Discussion**
304   This experiment explored the effect of idle cropland of two crops, chili and rice, on the
305   cultivation of *S. rugosoannulata*. The results showed that this cultivation method significantly
306   increased the yield and amino acid content of *S. rugosoannulata* and significantly changed the
307   soil microbial community structure.Generally speaking, soil properties have a great influence on
308   mushroom cultivation [23]. Our experiments have proved that the two crop rotation ways have
309   significantly increased the total nitrogen content of the soil and promoted the growth of *S.*
310   *rugosoannulata*. From the results of our experiments, it was found that total nitrogen in the soil
311   before cultivation of *S. rugosoannulata* was significantly elevated after the harvest of both crops
312   compared to the open field. Therefore, we think that the increase of total nitrogen in soil may be
313   a critical factor in promoting the growth of *S. rugosoannulata*, which was also verified in the
314   experiments of Keyvan Esmaeilzadeh-Salestani et al., nitrogen in the soil is taken up and
315   transformed by ammonium transporters (AMTs) in plants and ultimately promote plant growth
316   [33].This is consistent with prior research findings. Due to the rise in microbial variety in rice-
317   planted fields, the ability of microbial nitrogen fixation has improved, resulting in an increase in

318 the soil's nitrogen content [34]. Also shown the same ability to manage soil microbes was pepper
319 as a crop rotation. After planting pepper, the relative number of bacteria and fungi in the soil
320 increased dramatically, resulting in an increase in the soil's nitrogen concentration [35]. Nitrogen
321 in the soil can be taken and transformed by plant roots into amino acids, structural proteins, and
322 genetic material, according to studies [36, 37]. This may explain why the amino acid content of
323 *S. rugosoannulata* in our experiment increased dramatically under two rotation patterns. We also
324 noticed that in addition to total nitrogen, the phosphorus content in soil was also significantly
325 increased due to the two rotation modes. The rice ecosystem had a huge impact on the
326 composition of soil microbial community structure, and the change of microbial community also
327 changed the phosphorus cycle in soil [38]. Increasing the soil's phosphorus concentration can
328 considerably boost spore production and the colonization rate of arbuscular mycorrhizal fungi
329 [39]. In our experimental results, both phosphorus levels were significantly higher in the soils of
330 agricultural fields planted with chili and rice. It is due to this mechanism that a high nitrogen
331 content in the soil can promote the growth of *S. rugosoannulata* and improve their quality and
332 yield. Other than that, this may be due to the fact that different farming patterns regulates the
333 structure of soil microbial community and promotes the conversion of phosphorus to available
334 phosphorus. We discovered a significant increase in soil nutrient content in paddy fields, which
335 may be a result of significant changes in microbial communities within the paddy fields'
336 anaerobic environment [40]. In addition, the genome sequencing of *S. rugosoannulata* revealed
337 that it possesses powerful bioremediation and lignin degradation capabilities, so the secondary
338 metabolites that can be produced can significantly improve soil nutrient levels [41]. It has been
339 demonstrated that the used mushroom stock remaining after the fruiting bodies have been
340 harvested adds many nutrients to the soil [8].
341 There are many ways in which soil rhizosphere microorganisms play a role in promoting crop
342 growth[42], for example, fix nutrients in the soil, improve crop utilization, metabolize plant
343 auxins and avoid pathogen infection [43]. According to our and previous research, the
344 composition and structure of soil microorganisms are associated with soil nutrient levels [44].
345 Keyvan Esmaeilzadeh-Salestani et al. ' s experiment also proved our conjecture. Their research
346 revealed that increasing soil organic carbon content was advantageous for increasing microbial
347 diversity, whereas rotation was more favorable to preserving soil organic matter and maintaining
348 microbial activity [45]. Therefore, we investigated the effects of *S. rugosoannulata* on soil
349 microbial structure under two rotation modes. Studies have found that *Chloroflexi* has the
350 genomic potential of using formate, which can rapidly carry out the carbon cycle and improve
351 soil organic matter and nutrients [46]. Therefore, we speculate that in our experiment, it may be
352 due to the increase in Chloroflexi abundance, thereby improving soil fertility and ultimately
353 promoting the growth of *S. rugosoannulata*. *Sphingomonas* has outstanding performance in
354 remediate soil pollution and promoting plant growth. Recent studies have found that
355 *Sphingomonas* can produce plant growth hormones such as gibberellin and indole acetic acid,
356 which can promote plant growth [47]. Our experiment also found that the abundance of
357 *Sphingomonas* in agricultural soils after planting chili and rice significantly increased, which

358 also verified that *Sphingomonas* can promote crop growth. *Gemmatimonas* is a phototrophic
359 bacteria based on chlorophyll [48]. Light can provide more energy for its growth and
360 metabolism, thereby increasing the content of soil organic matter [49]. Similarly, *Gemmatimonas*
361 was significantly increased in the Rice-*S. rugosoannulata* group, which promoted the growth of
362 *S. rugosoannulata.* In our experiment, *Acidobacteria* increased significantly in the Rice-*S.*
363 *rugosoannulat* group. Genomic studies have shown that *Acidobacteria* has the ability to convert
364 inorganic nitrogen into organic compounds. In addition, *Acidobacteria* can secrete extracellular
365 polysaccharides, which can change the properties of rhizosphere soil and promote crop nutrient
366 absorption. It may be that Acidobacteria regulates soil properties and ultimately promotes the
367 growth of *S. rugosoannulat.* By fungal sequencing analysis we found that the expression
368 abundance of *Mortierella* in Chili- *S. rugosoannulata* and Rice- *S. rugosoannulataI* group
369 significantly increase. Studies have shown that *Mortierella* can be used as a biological control
370 agent to reduce the occurrence of root rot [50]. In addition, *Cladosporium*, which is significantly
371 increased in Chili- *S. rugosoannulata* and Rice- *S. rugosoannulataI* group, can metabolize and
372 release two volatile organic compounds (2-methyl-butyraldehyde and 3-methyl-butyraldehyde),
373 which can significantly enhance crop growth and roots development [51].In our experiment, it
374 also promoted the growth of *S. rugosoannulata*. After *S. rugosoannulata* was cultivated, the
375 significant increase in *Stropharia* abundance in Chili- *S. rugosoannulata* and Rice- *S.*
376 *rugosoannulataI* group should be due to the fact that the yield of *S. rugosoannulata* in the soil
377 was significantly higher than that of the NC group. *Mycotoxins* is a fungus that can produce
378 toxins [52].
379 Through gene function analysis of bacteria, we found that the two-component system, Microbial
380 metabolism in diverse environments, was significantly enriched in Chili-*S. rugosoannulata* and
381 Rice-*S. rugosoannulata* groups. Bahn et al. find the two-component system can cascades control
382 fundamental cellular functions related to fungal growth [53]. Most two-component system-
383 related signaling proteins are essential for fungal growth [54]. Microbial metabolism-related
384 genes regulate bacterial metabolism and promote bacterial production of growth-promoting
385 factors to regulate crop growth [55]. This also indicates that the soil microbial community
386 structure was greatly regulated after cultivation of S. rugosoannulata, and that the microbial
387 growth-promoting function was improved. Thus, we believe that mushroom cultivation in winter
388 on vacant cropland can not only improve its quality and yield, but also increase soil microbial
389 diversity, improve soil fertility, and lay a good foundation for a new round of crops in the
390 coming year.

391 **Conclusions**

392 In this study, we systematically investigated changes in soil microbial communities in three
393 cropping patterns: blank cropland, Chili-*S. rugosoannulata* and Rice-*S. rugosoannulata*. The
394 results showed that the two crop rotation modes, Chili- *S. rugosoannulata* and Rice- *S.*
395 *rugosoannulata*, significantly increased the yield, quality, and amino acid content of *S.*
396 *rugosoannulata,* improved soil quality. Notably, S. rugosoannulata cultivation significantly
397 increased the abundance of beneficial microorganisms such as *Chloroflexi*, *Cladosporium* and

398 *Mortierella* and reduce the abundance of *Botryotrichumin* and *Archaeorhizomyces*. This leads us
399 to believe that *S. rugosoannulata* cultivation in cropland can improve soil properties, regulate the
400 community structure of soil microorganisms, increase the expression abundance of beneficial
401 organisms and ultimately improve the *S. rugosoannulata* yield and lay a good foundation for a
402 new round of crops after this edible mushroom cultivation.

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406

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546

548

Figure 1

The quality and yield of *S. rugosoannulata* in different croplands.

(a) *S. rugosoannulata* produced in the NC group; (b) *S. rugosoannulata* produced in the Chili-*S. rugosoannulata* group; (c) *S. rugosoannulata* produced in the Rice-*S. rugosoannulata* group; (d) Yield per mu of *S. rugosoannulata* in different croplands. Data are mean ± SD (n = 3) and analyzed by one-way ANOVA. * $p < 0.05$.

NC   **Chili-*S. rugosoannulata***   **Rice-*S. rugosoannulata***
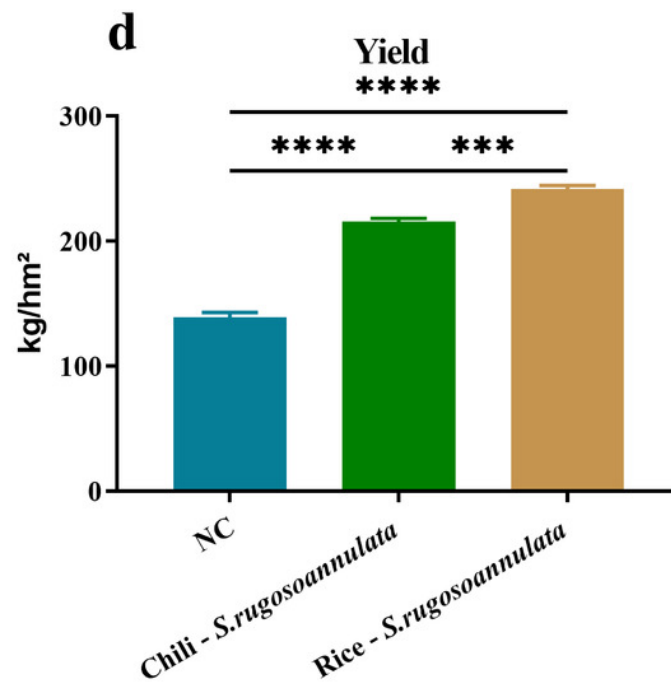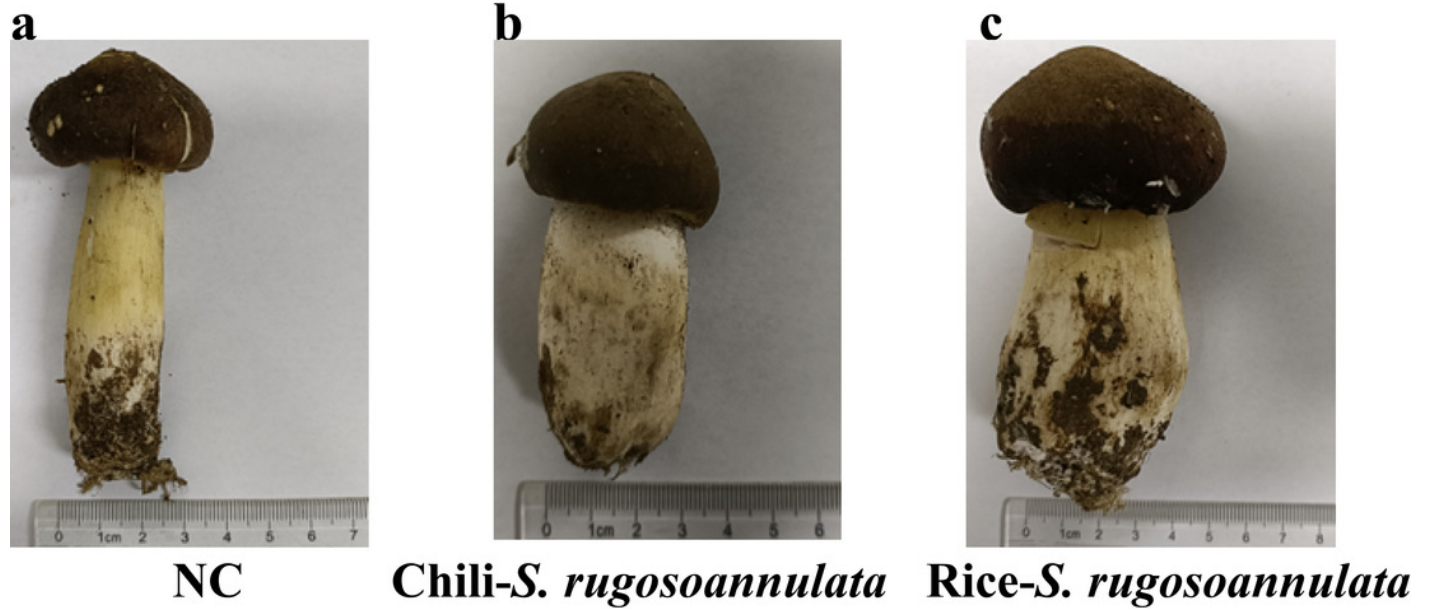
Figure 2

Soil properties in different croplands.

(a) The total nitrogen content of Soil before, during, and after cultivating *S. rugosoannulata* under different rotation patterns; (b) The available phosphorus content of Soil before, during, and after cultivating *S. rugosoannulata* under different rotation patterns; (c) The organic carbon content of Soil before, during, and after cultivating *S. rugosoannulata* under different rotation patterns; (d) The available potassium content of Soil before, during, and after cultivating *S. rugosoannulata* under different rotation patterns; (e) The soil moisture before, during, and after cultivating *S. rugosoannulata* under different rotation patterns. Data are mean $\pm$ SD (n = 3) and analyzed by one-way ANOVA. * $p < 0.05$.
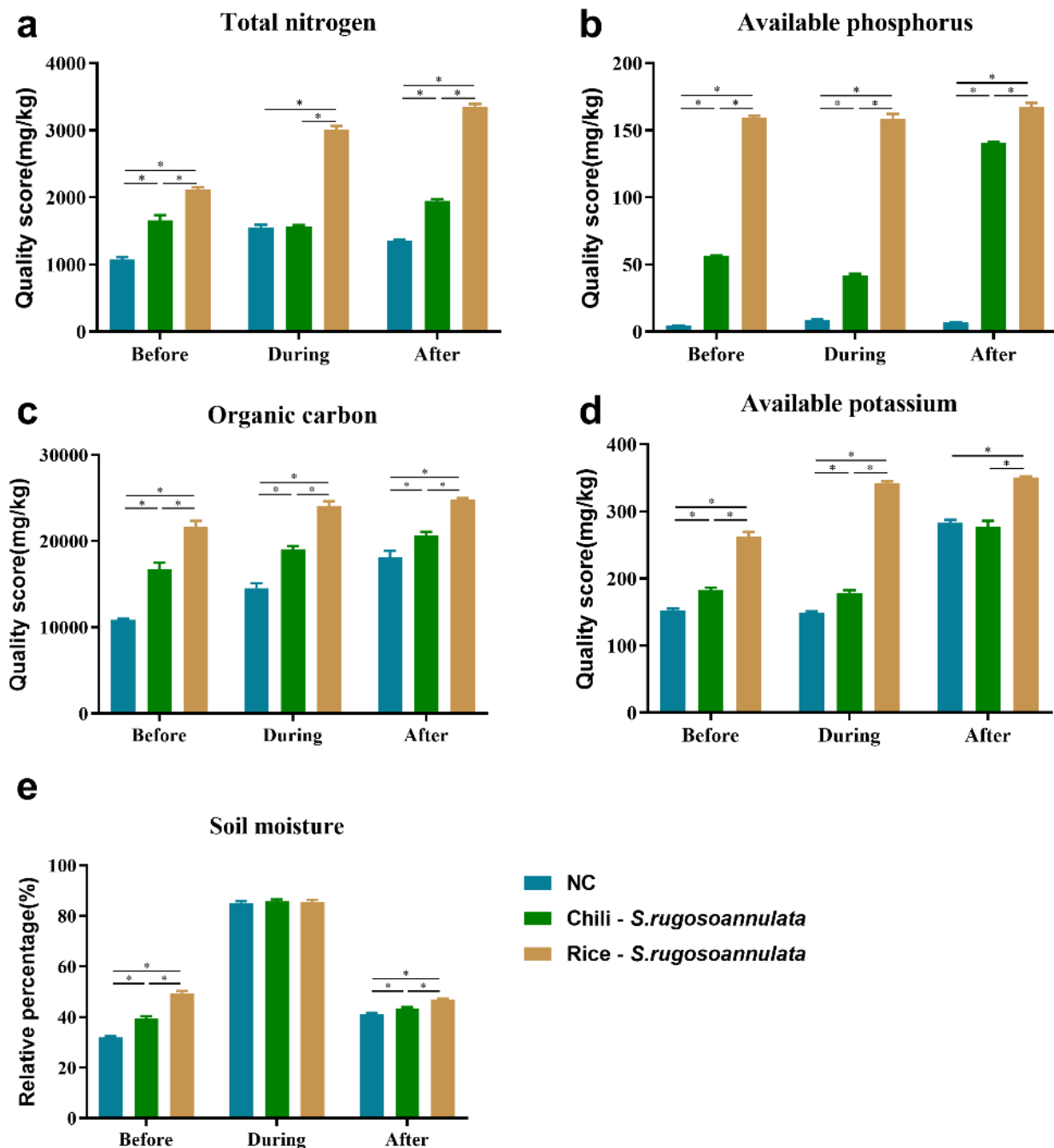
Figure 3

α-diversity of soil rhizosphere microorganisms after cultivating *S. rugosoannulata* in different croplands.

(a) ACE index; (b) Chao index; (c) Shannon index; (d) Simpson index of soil rhizosphere microorganisms after cultivating *S. rugosoannulata.*
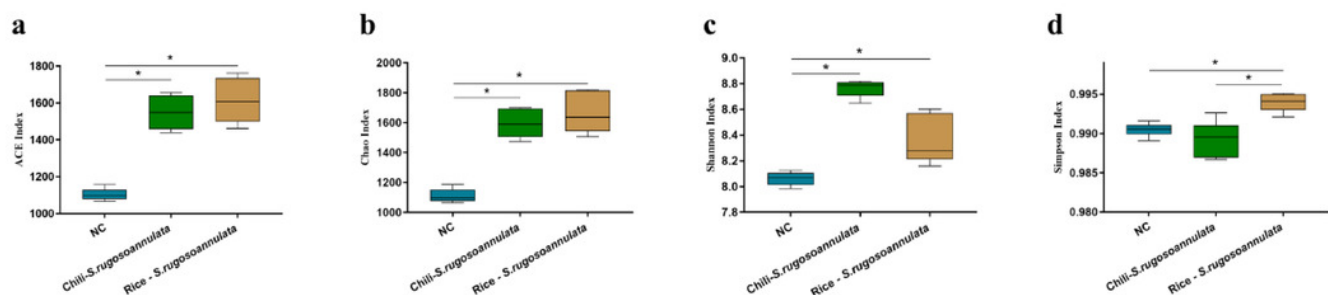
Figure 4

Rhizosphere bacterial community structure at phylum level after cultivating *S. rugosoannulata* in different croplands.

(a) Relative abundance of rhizosphere microbiota at the phylum level after cultivating *S. rugosoannulata*; (b) percentage of *Proteobacteria* in each sample from the three groups after cultivating *S. rugosoannulata*; (c) percentage of *Bacteroidetes* in each sample from the three groups after cultivating *S. rugosoannulata*; (d) percentage of *Chloroflexi* in each sample from the three groups after cultivating *S. rugosoannulata*; (e) percentage of *Actinobacteria*in each sample from the three groups after cultivating *S. rugosoannulata*; (f) Relative abundance of rhizosphere microbiota at the genus level after cultivating *S. rugosoannulata*; (g) percentage of *Sphingomonas* in each sample from the three groups after cultivating *S. rugosoannulata*; (h) percentage of *Candidatus_Solibacter* in each sample from the three groups after cultivating *S. rugosoannulata*; (i) percentage of *Granulicella* in each sample from the three groups after cultivating *S. rugosoannulata*; (j) percentage of *Gemmatimonas* each sample from the three groups after cultivating *S. rugosoannulata*. Data are mean ± SD (n = 6) and analyzed by one-way ANOVA. *$p < 0.05$
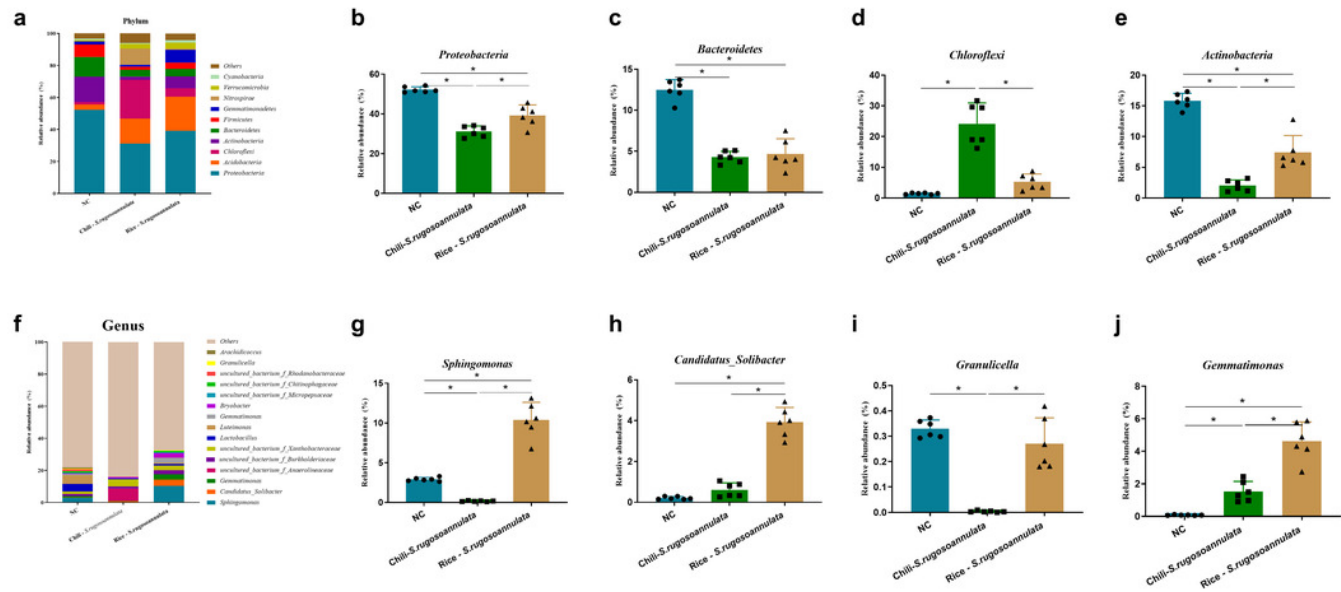
Figure 5

Rhizosphere fungal community structure at phylum level after cultivating *S. rugosoannulata* in different croplands.

(a) Relative abundance of rhizosphere fungal at the genus level after cultivating *S. rugosoannulata*; (b) percentage of *Ascomycota* in each sample from the three groups after cultivating *S. rugosoannulata*; (c) percentage of *Chytridiomycota* in each sample from the three groups after cultivating *S. rugosoannulata*; (d) percentage of *Basidiomycota* in each sample from the three groups after cultivating *S. rugosoannulata*; (e) percentage of *Mortierellomycota* each sample from the three groups after cultivating *S. rugosoannulata*; (f) Relative abundance of rhizosphere fungal at the genus level after cultivating *S. rugosoannulata*; (g) percentage of *Stropharia* in each sample from the three groups after cultivating *S. rugosoannulata*; (h) percentage of *Myrmecridium* in each sample from the three groups after cultivating *S. rugosoannulata*; (i) percentage of *Chaetosphaeria* in each sample from the three groups after cultivating *S. rugosoannulata*; (j) percentage of *Scytalidium* each sample from the three groups after cultivating *S. rugosoannulata*. Data are mean ± SD (n = 6) and analyzed by one-way ANOVA. *$p < 0.05$
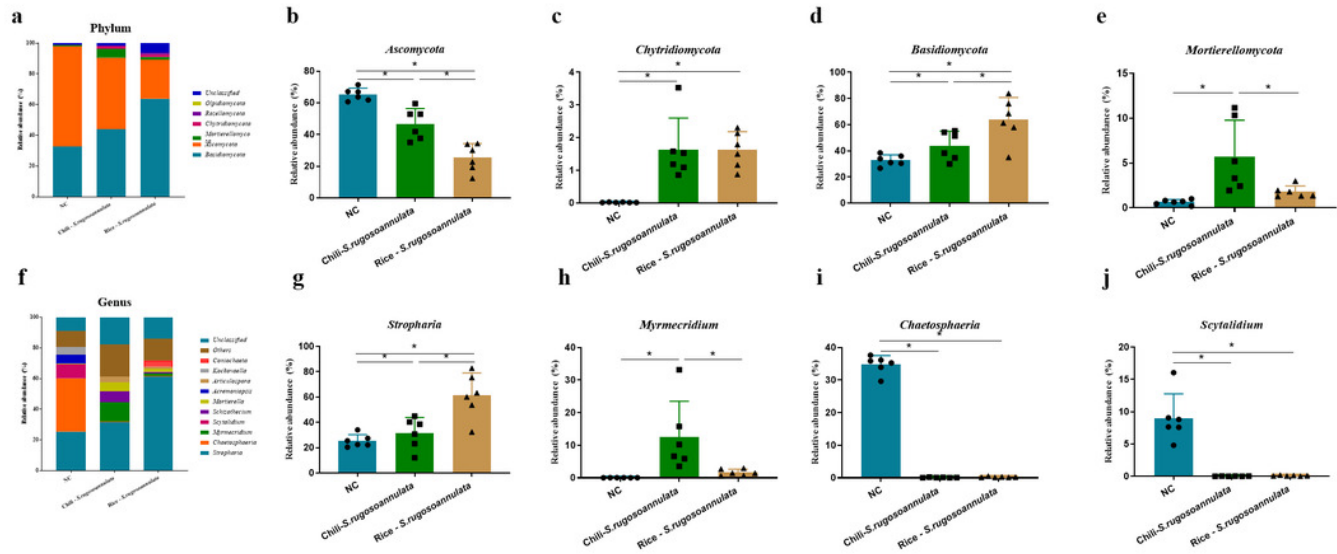
Figure 6

RDA analysis and correlation analysis of bacteria and fungi at the phylum level.

(a) RDA analysis between soil rhizosphere bacteria and environmental factors. (b) RDA analysis between soil rhizosphere fungi and environmental factors. (c) Correlation analysis of soil rhizosphere bacteria at phylum level and *S. rugosoannulata* amino acid content *S. rugosoannulata.* (da) Correlation analysis of soil rhizosphere fungi at phylum level and *S. rugosoannulata* amino acid content *S. rugosoannulata.*
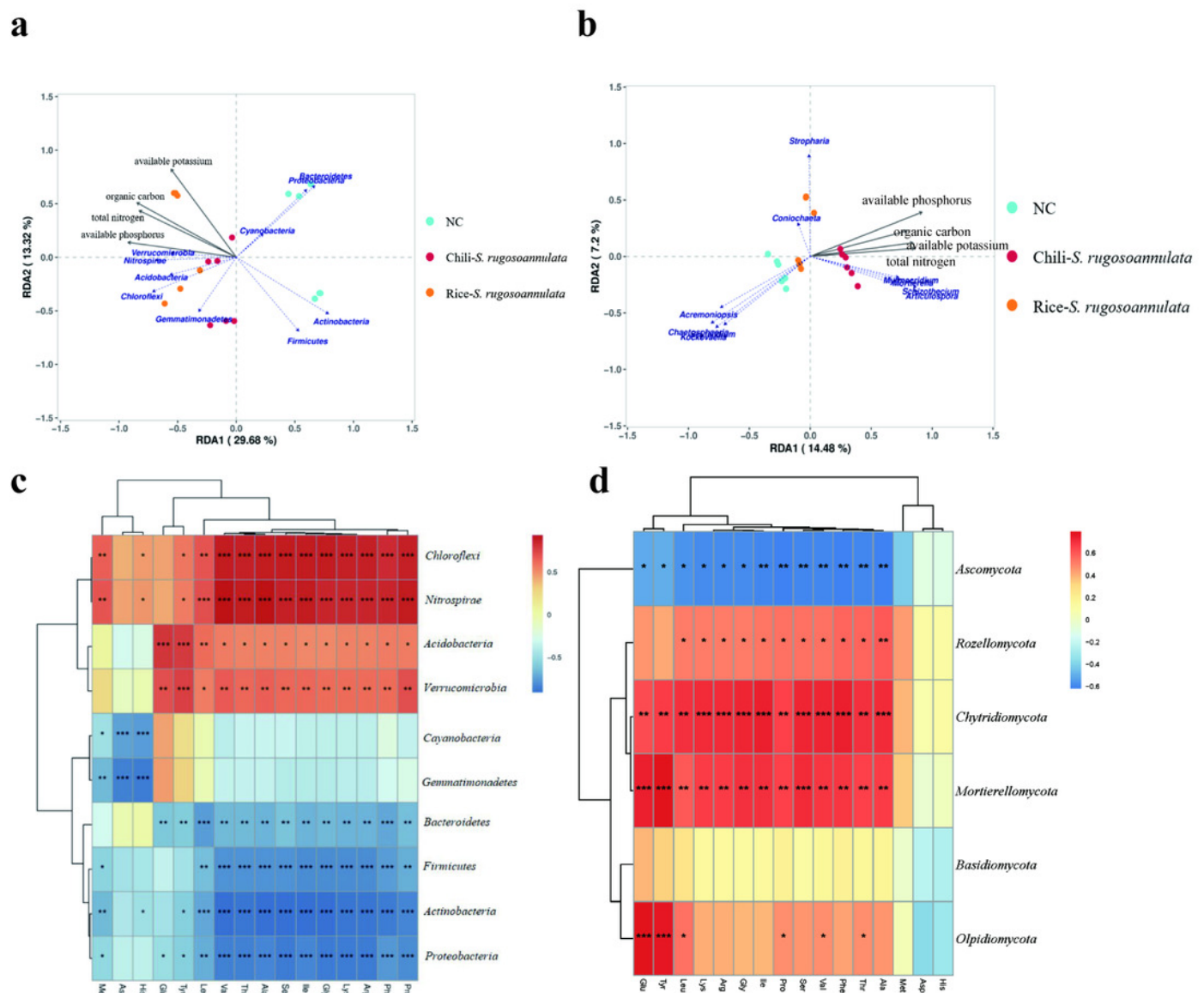
Figure 7

Prediction of microbial Tax4Fun functional genes in different croplands.

(a)Differential bacterial functional genes between NC group and Chili-*S. rugosoannulata* group. (b) Differential bacterial functional genes between NC group and Rice-*S. rugosoannulata* group. (c) Differential fungi functional genes between NC group and Chili-*S. rugosoannulata* group. (d) Differential fungi functional genes between NC group and Rice-*S. rugosoannulata* group.

a

	95% confidence intervals	

NC   Chili-*S.rugosoannulata*

Two-component system — 2.19e-08
Biosynthesis of secondary metabolites — 1.80e-07
Biosynthesis of antibiotics — 3.04e-07
Carbon metabolism — 3.98e-07
Metabolic pathways — 1.43e-06
Microbial metabolism in diverse environments — 2.21e-06
Biosynthesis of amino acids — 1.55e-04
Fatty acid metabolism — 1.02e-03
Quorum sensing — 1.09e-03
ABC transporters — 1.02e-02

Mean proportion (%)   0.0   13.5

Difference between proportions (%)   -1.0   -0.5   0.0   0.5

p-value (corrected)

b

	95% confidence intervals	

NC   Rice-*S.rugosoannulata*

Biosynthesis of antibiotics — 1.45e-11
Biosynthesis of secondary metabolites — 1.10e-10
Quorum sensing — 1.54e-09
Metabolic pathways — 2.68e-07
ABC transporters — 1.05e-06
Microbial metabolism in diverse environments — 1.18e-06
Carbon metabolism — 1.35e-06
Fatty acid metabolism — 3.41e-05
Biosynthesis of amino acids — 9.19e-05
Two-component system — 1.64e-03

Mean proportion (%)   0.0   13.5

Difference between proportions (%)   -0.5   0.0   0.5   1.0

p-value (corrected)

c

	95% confidence intervals	

NC   Chili-*S.rugosoannulata*

Endophyte — 1.96e-06
Litter Saprotroph — 1.10e-05
Wood Saprotroph — 1.45e-05
Dung Saprotroph — 1.48e-03
Undefined Saprotroph — 3.26e-03
Soil Saprotroph — 5.51e-03
Plant Parasite — 6.09e-03
Plant Pathogen — 2.87e-02
Animal Parasite — 3.92e-02
Plant Saprotroph — 4.54e-02

Mean proportion (%)   0   29

Difference between proportions (%)   -20   -10   0   10

p-value (corrected)

d

	95% confidence intervals	

NC   Rice-*S.rugosoannulata*

Undefined Saprotroph — 7.25e-07
Plant Parasite — 4.42e-06
Dung Saprotroph — 6.42e-06
Soil Saprotroph — 6.50e-06
Endophyte — 9.84e-06
Litter Saprotroph — 1.17e-05
Wood Saprotroph — 7.41e-05
Plant Pathogen — 3.05e-02

Mean proportion (%)   0.0   28.8

Difference between proportions (%)   -15   -10   -5   0   5   10

p-value (corrected)

The amino acid content of *S.rugosoannulata* in different croplands*.*

Amino acid (µg/mg)	NC	Chili-*S.rugosoannulata*	Rice-*S.rugosoannulata*
Asp	1.670±0.039[b]	1.589±0.053[b]	2.060±0.041[a]
Thr	0.901±0.023[c]	0.968±0.017[b]	1.128±0.050[a]
Ser	0.892±0.025[c]	0.984±0.024[b]	1.091±0.038[a]
Glu	2.362±0.222[b]	3.231±0.036[a]	3.067±0.085[a]
Gly	0.715±0.018[c]	0.813±0.029[b]	1.071±0.021[a]
Ala	0.975±0.008[c]	1.367±0.102[b]	1.502±0.030[a]
Val	0.972±0.021[c]	1.104±0.034[b]	1.252±0.022[a]
Met	0.313±0.019[b]	0.308±0.020[b]	0.480±0.042[a]
Ile	0.667±0.021[c]	0.801±0.015[b]	0.905±0.022[a]
Leu	1.225±0.038[b]	1.487±0.196[a]	1.557±0.053[a]
Tyr	0.538±0.022[b]	0.620±0.020[a]	0.594±0.015[a]
Phe	0.855±0.009[b]	0.940±0.023[b]	1.043±0.082[a]
His	2.967±0.085[c]	2.235±0.035[b]	5.881±0.159[a]
Lys	0.960±0.025[c]	1.078±0.031[b]	1.388±0.026[a]
Arg	0.746±0.015[b]	0.783±0.016[b]	0.921±0.036[a]
Pro	0.486±0.015[b]	0.558±0.037[a]	0.599±0.015[a]

1   [a, b, c] mean values with different letters are significantly different from each other ($p < 0.05$).