Bacterial community diversity, lignocellulose components, and histological changes in composting using agricultural straws for *Agaricus bisporus* production

Tingting Song [1], **Yingyue Shen** [1], **Qunli Jin** [1], **Weinlin Feng** [1], **Lijun Fan** [1], **Guangtian Cao** [Corresp., 2], **Weiming Cai** [Corresp. 1]

[1] Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, hanzghou, zhejiang, China

[2] China Jiliang University, College of Standardisation, Hangzhou, Zhejiang, China

Corresponding Authors: Guangtian Cao, Weiming Cai
Email address: 15a1903025@cjlu.edu.cn, caiwm0527@126.com

Agricultural straws (AS) may serve as potential base-substances in the production of *Agaricus bisporus*. Six AS that occur across China were investigated in a two-stage composting experiment; lignocellulose components, AS morphology, and the effects of different AS on mushroom yields from 2015–2017 were examined. In addition, microbial biodiversity and their impact on substrate degradation were studied using 16S gene sequencing based on six different AS on the 3rd (I.F), 6th (I.S), and 10th (I.T) day of Phase I, and Phase II (II). Results showed that the six different AS exhibited differences in the progression of degradation under the same compost condition; the wheat straw, rice straw, and cotton straw induced a significantly higher mushroom yield than did the others (*P*<0.05); *Thermobispora*, *Thermopolyspora,* and *Vulgatibacter* genera may play an important role in the different AS degradations. According to our experiments, we can adjust formulations and compost methods to obtain high-yield mushroom compost based on different AS in the future.

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3 Tingting Song[1], Yingyue Shen[1], Qunli Jin[1], Weilin Feng[1],Lijun Fan[1], Guangtian Cao[2*], Weiming
4 Cai[1*]
5 [1]Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021,
6 Zhejiang, China;
7 [2]College of Standardisation, China Jiliang University, Hangzhou 310018, Zhejiang, China;
8
9 *Corresponding author:
10 Cao Guangtian, College of Standardisation, China Jiliang University, Hangzhou, Zhejiang,
11 310018,   People's Republic of China; E-mail: 15a1903025@cjlu.edu.cn; Tel: 86-571-
12 8618758235285
13 Weiming Cai, Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou,
14 Zhejiang, 310021, People's Republic of China; E-mail: CaiWM527@126.com; Tel: 86-571-
15 86404017

Abstract

Agricultural straws (AS) may serve as potential base-substances in the production of *Agaricus bisporus*. Six AS that occur across China were investigated in a two-stage composting experiment; lignocellulose components, AS morphology, and the effects of different AS on mushroom yields from 2015–2017 was examined. In addition, microbial biodiversity and their impact on substrate degradation were studied using 16S gene sequencing based on six different AS on the 3rd (I.F), 6th (I.S) and 10th (I.T) day of Phase I and Phase II (II). Results showed that the six different AS exhibited differences in the progression of degradation under the same compost condition; the wheat straw (WS), rice straw (RS), and cotton straw (CS) induced a significantly higher mushroom yield than did the others ($P<0.05$); *Thermobispora*, *Thermopolyspora,* and *Vulgatibacter* genera may play an important role in different AS degradation. According to our experiments, we can adjust formulations and compost methods to obtain high-yield mushroom compost corresponding different AS in the future.

Keywords: *Agaricus bisporus*; agricultural straw; lignocellulose; composting; histological changes; 16S rRNA sequencing; mushroom yield

Introduction

Agricultural straws (AS) are organic agricultural residues produced at a global rate of $2–4 \times 10^9$ t per year, which is 1.4 times the annual crop yield (Smil 1999). AS comprise mainly of lignocellulosic biomass, most of which is used for combustion in China(Sheng et al. 2018). Until 2018, the amount of AS had reached 886 million tons in China. AS waste is rich in macromolecular organic matter, including cellulose, hemicellulose, starch, and lignin. Many techniques have been applied to the utilization of AS waste resources. Among these methods, mushroom cultivation is one of the most efficient and economical biotechnological process for converting lignocellulosic materials into high quality food. Rice straw and wheat straw have been used for hydrogen production(Liu et al. 2015) and ethanol fermentation (Nair et al. 2017).

White button mushrooms (*Agaricus bisporus*) are well-known basidiomycete fungi, grown on substrate with a casing layer and produced in large quantities for human consumption (De Groot et al. 1998). In America and Europe, the wheat straw is a major traditional ingredients for *A. bisporus* cultivating (Straatsma et al. 2000). In China, rice straw is widely available, and has formed the major material for *A. bisporus* cultivation in recent decades(Gong 2011). There are three distinct phases of the compost production and preparation(McGee 2018): Phases I and II form the aerobic fermentation processes (Simsek et al. 2008), and Phase III form inoculation and mycelium growth processes (Patyshakuliyeva et al. 2015). In general, Phase I periods of about 5–7 and 15–21 days are used in Europe and China, respectively. The compost temperature stays at approximately 80 °C due to the growth of thermophilic microorganisms (Zhang et al. 2019). Subsequently, a pasteurization process (Phase II) is performed at 45–50 °C for about 4–9 days until the ammonia level decreases to a level that is non-toxic to *A. bisporus* mycelia. The compost preparation is achieved by manipulating the natural succession of microorganisms present in the raw materials. The microbial populations, substrate compositions, and the physical states of the materials change during Phases I and II of this

56   process.

57   The bacterial community of the compost is highly diverse, and exhibits dynamic changes
58   in response to the quality of the compost (Székely et al. 2009; Zhang et al. 2014; Siyoum et
59   al. 2016; McGee et al. 2017). The substrates are self-heated after mixing and wetting in Phase I
60   compost and are characterized by high microbial activity (Miller et al., 1989), while aerobic
61   microorganisms dominate the fermentation process because of controlled temperature
62   conditions in Phase II compost(Straatsma et al. 1994). For the past decades, several techniques
63   have been used for studying of microbial communities at different stages, including community-
64   level physiological profile analysis, phospholipid fatty acid analysis, and environmental DNA
65   amplification-based methods(Carpenter-Boggs et al. 1998; Dees and Ghiorse 2001; Tiquia and
66   Michel 2002;Helgason et al. 2010; Takaku et al. 2006). The development of next-generation
67   sequencing technologies has provide a more reliable method for the analysis of the depth and
68   composition of microbial communities.

69   In order to make full use of AS across the different areas of China, six major straws and
70   residues, including wheat straw (WS), rice straw (RS), cotton straw (CS), corncob (CC), corn
71   straw (C), and bagasse (B), were selected as the representative substrates to cultivate *A.
72   bisporus,* following the initial screening of various materials. Then, physicochemical properties,
73   histological changes, and the production properties of the different *A. bisporus* compost
74   materials were compared. Additionally, the bacterial community structure of four-stage
75   composting, based on six different AS, was analyzed. The data obtained by our experiment
76   may help understand the optimal composting conditions corresponding to certain AS
77   formulations. The efficient utilization of different AS from different areas as substrates for *A.
78   bisporus* cultivation has the potential to reduce the cost of transporting raw materials and
79   increase farming profits in China.

80   **Materials and Methods**

81   **Mushroom strain**

82   *The A. bisporus* strain A106 was obtained from our lab and cultured at 25°C on potato dextrose
83   agar (PDA) medium for further experiment. Wheat grains (purchased from a local grocery store)
84   were used to prepare *A. bisporus* spawn as described by Cheng (1999).

85   **Composting and sampling**

86   Composting was conducted in the fermentation room of our experimental base located in
87   Hangzhou, Zhejiang province, between 2015 and 2017. The composting windrows (5 × 2.0 × 1.8
88   m) each contain a 600 kg mixture consisting of Agriculture straw, rapeseed cake, $CaSO_4 \cdot 2H_2O$,
89    $Ca(H_2PO_4)_2 \cdot H_2O$, $(NH_4)_2SO_4$, and urea (CON_2H_4), with the formulations of ingredients listed in
90   S1.

91   Before composting stage, the raw material were mixed and wetted by manual spraying to
92   adjust the moisture around 75%, and ~200 g samples (M) were collected in triplicate. At Phase I
93   of the 3th (I.F), 6th (I.S), and 10th (I.T) day, the windrows were turned on to keep the
94   concentration of O_2 sufficient and enhance the composting process. $CaCO_3$ and water were added
95   manually to maintain the pH (6−8) and moisture content (60−70%) during the turning(data in
96   S2). Phase II (II), consisting of 6 days was characterized by a rapid increase in temperature up to
97   60°C for 8−9 h, followed by stabilization of the compost temperature to 45−50°C for 5 days, and
98   gradual cooling to 25°C. Samples at four different stage(~200 g each) were collected and dried
99   using a vacuum freeze dryer as previously described(Song et al. 2014), then stored at −80°C for
100 further analysis.

Analysis of physical and chemical properties

102 Throughout Phase I and II, the temperature of the tunnel was monitored by tubular mercurial
103 thermometers continuously placed in the middle of the compost (Kai Longda Instrument Co.,
104 Tianjin, China).The water content was measured by water analyzer (Ohaus, MB25) with 5 g sub-
105 samples. The pH values were determined with an electronic pH meter (Mettler-Toledo
106 Instruments Co., Ltd., Shanghai, China) using 10% (w/v) sample suspensions (in distilled water)
107 were used. The total nitrogen (N) and carbon (C) levels were assayed by the methods of Kjeldahl
108 (Bremner and Mulvaney 1982) and $K_2Cr_2O_3$ oxidation (Fan 2007), and then C/N ratios were
109 calculated. In addition, the fiber profiles (including crude fiber of the cell wall, cellulose,
110 hemicellulose, and acid digestible lignin [ADL]) were analyzed as described by Van Soest et al.
111 (1991). The percentage of dry matter losses was calculated according to the following equations:
112 Dry matter loss (%) = (the dry matter weight after Phase II / the dry matter of initial formulation)
113 × 100%.

AS stem sub-samples for transmission electron microscopy

115 The sub-stem samples (3 cm long) were obtained from dried samples of six different AS
116 composts collected after Phase II. Each sample was prepared and subjected to the method
117 described by Song et al. (2014). The samples were examined using a JEM-1230 transmission
118 electron microscope(TEM).

Determination of mushroom yields

120 The mushroom yields were recorded from 2015–2017 for three years. The pawning rate of six
121 different pasteurized compost was 1% (w/w) . Then, the spawned compost was divided into 15.0
122 kg samples (wet weight, moisture content ~65%) and transferred to a 45 × 35 × 24 cm plastic
123 cultivation boxes. After growing the mycelia at 22–25°C for 25 days, the casing soil was
124 overlaidto initiate primordium formation. Fruit bodies in different AS composts were harvested,
125 and the weights of the total fresh mushroom yields (TY, g/kg substrate) during the harvesting
126 period, as well as the 1–3 flushes (T3) of fruit bodies were recorded. Biological efficiency (BE)
127 values were calculated by the ratio of fresh mushroom weight to dry substrate weight. All

128 experiments in each year included 10 replicates for each treatment, and the yields of mushroom
129 cultured with different AS-based substances were calculated by the average of the three-year
130 data from 2015–2017.

High-throughput sequencing of 16S rRNA

132 Total genomic DNA was extracted from 1.0 g mixture of AS (3 collection points in 2017), using
133 the Power Soil DNA Extraction Kit (Mo Bio Laboratories Inc., Carlsbad, CA, USA) as per the
134 manufacturer's instructions. The 16S rRNA amplicon sequencing was performed according to
135 our previous study (Cao et al., 2018); briefly, sequencing was performed on a Illumina MiSeq
136 platform at Novogene Cooperation (Beijing, China) with a HiSeq 2500 (PE250) sequencing
137 system, using the specific primer pair of 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R
138 (5′-GGACTACHV GGGTWTCTAAT -3′), targeting the V3-V4 hypervariable region. The data
139 that supporting this study are openly available in the NCBI GenBank(BioProject accession
140 number:PRJNA664798).

Statistical analysis

142 The data are recorded as the means of replicates with standard error(±SE). SPSS 16.0
143 software was used to analyze the data through one-way and two-way ANOVA, and $P<$
144 0.05 were considered to indicate significant differences using the Tukey test. For
145 bioinformatics analysis, as per our previous study (Cao et al. 2018), the similarity or
146 difference between the different sequencing libraries was assessed by the α- (Shannon and
147 Simpson indices) and β-diversity parameters, with the former analyzed by the Wilcoxon signed-
148 rank test and the latter by the weighted UniFrac analysis (QIIME). Principal coordinate analysis
149 (PCoA) and non-metric multidimensional scaling (NMDS) plot analysis were performed using
150 the R software (Version 2.15.3). Linear discriminant analysis with effect size (LEfSe) tool was
151 accessed online at http://huttenhower.sph.harvard.edu/galaxy/. The student t-test was used to
152 analyze the mean difference between two samples of bacterial strains at the genus level for the
153 three stages of Phase I and II, respectively.

Results

Temperature change in the fermentation chamber from 2015-2017

156 Figure 1 shows that the mean compost temperatures in the fermentation chamber fluctuated daily
157 between 40.1 and 72.8ºC during the composting period from 2015 to 2017. As expected, the
158 compost temperatures decreased rapidly on the 4th, 6th and 9th days due to turning. We found that
159 the temperature was maintained at approximately 65-70°C over the first 3 days, decreased from
160 70°C to 65°C on days 5–6 and 8–9 respectively, and then finally to approximately 55°C from
161 day 13 to the end of the trial. Because this trial was performed in temperature-controlled tunnel,
162 there was no dramatic difference in temperature in the fermentation chamber between from
163 2015–2017; this enabled stable composting over the three-year period.

164   **Physical characteristics of AS-based substances**

165   After the two phases of composting, the C/N ratios of the initial formulations of six different AS-
166   based substances, which were 29.6-32.8:1, decreased to final 15.1-19.2:1. There was no
167   significant difference in the C/N ratios among the different AS-based substances across all stages
168   (Figure 2A). The data showed no remarkable difference in the crude fiber content among all
169   samples at all time points apart from CS , which had a significantly higher crude fiber content
170   than WS in Phase II (Figure 2B). The data showed that the rice straw (RS)-based substrate had a
171   much greater nitrogen content than did the wheat straw (WS) and corncob (CC) based substrates
172   in the initial formulation stage. Moreover, RS had a significantly higher nitrogen composition
173   than did CC in the Phase I.F , while both cotton straw (CS) and CC had a significantly higher
174   nitrogen composition than bagasse (B) in Phase II (Figure 2C).

175   Furthermore, the percentages of cellulose, hemicellulose, and ADL in all phases were
176   examined(Figure 3). After Phase II, all substances sampled had significantly less hemicellulose
177   ($P < 0.05$) than that in the material phase, and samples of CS, WS, RS, and CC all had
178   significantly less cellulose ($P < 0.05$); no significant differences were found in the ADL
179   percentages across all samples (Figure 3B, C, and D). The dry matter loss was also analyzed at
180   the end of the trial, and we found that the CC had the lowest percentage loss, while CS and B had
181   the highest.

182   **TEM scanning of AS morphology**

183   The changes in AS structure from the material phase and Phase II were revealed by TEM
184   scanning, as shown in Figure 4. Compared to the straw materials of CS, CC, C, and B (Figure 4A,
185   J, I, and K), the cellular structure of straw in Phase II (Figure 4B, H, J, and L) was dramatically
186   destroyed, with numerous erosion troughs and cracks found on the surface of pretreated straws.
187   This was particularly evident in the structures of C and CC, where the straw was severely
188   destroyed and no intact cell wall structures were observed. The cellular structure of WS and RS
189   appeared deformed to a certain extent (Figure 4 C&D, and E&F), which crimped cell wall
190   generation.

191   **Yields of mushroom cultured with AS-based substances from 2015-2017**

192   The mushroom yields cultured with AS-based substances from 2015-2017 are shown in Table 1
193   (the average of the three year period)and S3. According to the data analysis, There was no
194   difference among the there-years mushroom yields in TY($F_{2, 17}$=0.15 P=0.86), T3($F_{2, 17}$=0.56
195   P=0.59), and BE($F_{2, 17}$=0.77 P=0.49), indicating that the three-year production data are relatively
196   stable. From the TY, the CS (596.7 g/kg) substance was significantly higher than that in other
197   groups, with RS (569.9 g/kg), WS (553.8 g/kg), and CC (498.0 g/kg) being higher than C (370.5
198   g/kg) and B (318.7 g/kg). The T3 performance was the highest in CS (501.2 g/kg), WS(478.6
199   g/kg) and RS (481.9 g/kg), and the lowest in C and B. We next analyzed the BE index for
200   explaining the mushroom production characteristics of the different materials. The data showed

201 that the highest BE value obtained for CS, WS and RS was 84.4,83.5 and 86.8 respectively,
202 while C (63.7%) had the lowest value.

16S High throughput sequencing results

204 The six AS under the same composting conditions were sampled as replicates; changes in the
205 compost microflora community in different phases are shown in Figure 5 and 6. Approximately
206 1899 common operational taxonomic units(OTUs) were shared between all samples, while 1485,
207 1157, 1170, and 1024 specific OTUs were found in I.F, I.S, I.T, and Phase II, respectively
208 (Figure 5A). Firmicutes, Proteobacteria, and Actinobacteria were the three predominant phyla in
209 all samples (Figure 5B). In addition, Bacteroidetes dominated the samples of Phase I.F , and
210 Gemmatimonadetes dominated the Phase II (Figure 5C). The top 5 dominant genera of samples
211 in the Phase I.F and I.S were *Pseudoxanthomonas*, *Thermobispora*, *Thermopolyspora*,
212 *Thermobifida,* and *Thermobacillus*; *Thermobispora*, *Pseudomonas*, *Thermopolyspora*,
213 *Thermobifida,* and *Ruminiclostridium* were the top 5 genera in the PhaseI.T; and *Thermobispora*,
214 *Thermopolyspora*, *Thermobifida*, *Microbispora* and *Thermobacillus* were the top 5 genera of
215 Phase II. Moreover, the α-diversity showed that the Shannon and Simpson indices of I.S samples
216 were significantly higher than those in Phase II samples; no remarkable differences were found
217 among all other samples (Figure 5D and 5E). Both PCoA and NMDS analysis showed that the
218 samples of Phase II were distinctly separated from other samples of Phase I (Figure 5F and 5G);
219 NMDS analysis further revealed that I.F, I.S, and I.T samples were also separated from each
220 other. Samples of Phase I.F had a higher relative abundance of the genus *Pseudoxanthomonas*
221 than did those of Phase I.T (*P*=0.085) and II (*P*=0.072), as determined by the Wilcoxon signed-
222 rank test (Figure 5H). Samples of Phase I.F had a higher relative abundance of the genus
223 *Thermobifida* than did those of I.T (*P*=0.088) and II (*P*=0.065). Samples of Phase II had a higher
224 relative abundance of the genus *Thermobispora* than did those of I.F (*P*<0.05) and I.S (*P*=0.073).

225 LEfSe analysis identified the different microflora strains present within all samples (for
226 LDA>3.5, Figure 6A). The species of *Pseudoxanthomonas taiwanensis*, genera of *Bacillus* and
227 *Sphingobacterium*, and families of *Paenibacillaceae* and *Beijerinckiaceae* were enriched in
228 samples of Phase I.F. The family of *Rhizobiaceae* and genus of *Chelativorans* were enriched in
229 Phase I.S samples. The species of *Pseudomonas fragi* and *Chryseobacterium hominis* were
230 enriched in Phase I.T samples. The class of Acidimicrobiia was enriched in Phase II samples. A
231 ternary plot was used to investigate the different bacterial strains of Phase I samples at the
232 species level (Figure 6B), which showed that *Pseudoxanthomonas taiwanensis*, *Ammoniibacillus*
233 *agariperforans,* and *Ureibacillus thermosphaericus* dominated in I.F in comparison with that
234 in I.T and I.S; *Pseudomonas fragi*, *Clostridium* sp. TG60.81, and *Thermobispora bispora*
235 dominated in I.T compared to the other two Phase I groups. Comparing the dominant bacterial
236 strains, we found that the relative abundance of *Thermobispora* and *Thermopolyspora* was
237 higher in Phase II than in Phase I.

238 Moreover, the t-test was used to identify the different bacterial strains at the genus level
239 between Phase I and II, which revealed the relative abundance of *Thermobispora*,

240   *Thermopolyspora,* and *Vulgatibacter* to be significantly higher in Phase II than in Phase I.F
241   (Figure 6C). The samples of I.S had a significantly higher (*P*<0.05) relative abundance of
242   *Paenibacillus*, *Tepidimicrobium*, *Chelatococcus*, *Desulfotomaculum,* and *Flavobacterium* than
243   did those of Phase II (Figure 6D). The samples of I.T had significantly higher (*P*<0.05) relative
244   abundance of *Sphingobacterium* and *Defluviitalea* than did those of Phase II (Figure 6D).

245   **Discussion**

246   The production of edible fungi has emerged as a potential biological use of agricultural AS,
247   supported by low energy consumption and environmentally impact. Given the large variety of
248   agricultural waste likely to be generated across China, the focus is mainly on the most prominent
249   residues, such as rice straw, wheat straw, rye straw, and banana plant waste(Zhang et al. 2019).
250   Typically, AS are treated as lignocellulosic agricultural byproducts, which comprise stalks, stems,
251   and cobs. The use of agricultural straws in the edible fungi industry does not only take advantage
252   of the waste of natural resources and reduce the environmental pollution derived from burning
253   abandoned straws, but also has economic benefits (Lu et al. 2018). As a kind of white rot fungi,
254   *A. bisporus* can degrade the majority of AS; in China, RS and WS have been identified as an
255   important raw source for the substances of *A. bisporus* compost (Wang et al. 2016). In the
256   current study that ranged from 2015 to 2017, the TY, T3, and EB parameters showed that the
257   substances of RS, WS, and CS exhibited the highest mushroom yield, while C and B had poor
258   yields under the same compost condition.

259   A large number of bacteria and fungi play a key role in the production of *A. bisporus*
260   compost. They fermented AS into high quality substrate for *A. bisporus* by the thermophilic
261   composting process. Those microorganisms decompose the compounds, release ammonia and
262   then digest cellulose and hemicellulose into compost microbial biomass serves as the nutrition
263   source of the *Agaricus* mycelium(Kertesz et al. 2018). Sparling et al. (1982) reported that wheat
264   straw represents an important carbon source for *A. bisporus* cultivation, and that microorganisms
265   play a key role in the degradation process. Shen et al. (2009) reported that straw, containing
266   abundant carbon, nitrogen, phosphorus (P), potassium (K), and other nutrients, is a valuable
267   organic fertilizer and renewable resource. A study conducted by Wang et al. (2016) found that
268   the C/N ratio in RS compost decreased more rapidly than that in WS compost. It has also been
269   reported that the soil C/N and total N concentration are significantly increased during corn straw
270   application (Lu et al. 2015). Here, no significant differences in the C/N ratio and N amount were
271   observed; RS had a higher N ratio than did CC in the Phase I.F, whereas CS and CC had a higher
272   N ratio than did B in Phase II. The correlation analysis indicated that the N concentration in
273   mature compost seemed to be related to the mushroom yield (*r* = 0.78, *P* < 0.05).

274   Microbial degradation of various agricultural residues leads to changes in physical and
275   nutritional qualities, which can, for example, lead to changes in white rot fungi texture,
276   degradation of lignin, and conversion of complex polysaccharides into simple sugars (Sharma
277   and Arora 2015). Here, the data revealed no significant differences in the crude fiber of
278   substances between the initial formulation to I.T, and that in Phase II, CS had a significantly

279 higher crude fiber content than did WS. Moreover, we determined the percentage of cellulose,
280 hemicellulose, and ADL at the end of the study, revealing that after Phase II, CS, WS, RS, and
281 CC after Phase II all had significantly less cellulose than did the other materials, and all compost
282 samples had a significantly lower percentage of hemicellulose. Based on the above data, we
283 suspected that the substance type of the compost influenced the digestive efficiency of
284 lignocellulose, which may be related to the chemical composition, cellulose structure,
285 antinutrients, and microorganism strains of the compost. Based on our data pertaining to the 17-
286 day aerobic short-time fermentation, high N amount and crude fiber concentration, and low dry
287 matter loss ratios are necessary for high *A. bisporus* production. For the present composting
288 method, WS, RS, CC, and CS are probably better suited for *A. bisporus* production, while over-
289 fermentation, or the low N content of C and B will lead to poor production of *A. bisporus*.

290 Sheng et al. (2018) showed that the structure of pretreated RS was severely destroyed, and
291 more cracks and large holes were observed with the extension of fermentation time through
292 scanning electron microscopy(SEM). Shahryari et al. (2018) found that high intensity fungal
293 growth led to larger accessible surface area and structural destruction in the wheat straw
294 observed by SEM. Similarly, in this study, TEM analysis showed that the cellular structure was
295 destroyed by the degradation of *A. bisporus*. Interestingly, the destruction level of C and CC
296 straw cellulose were higher than those in B and RS. Apparently, treatments with microflora not
297 only improve the degraded biomass quality, but also simultaneously aid in the production of
298 various commercial enzymes, which was connected to edible fungal production(Sharma and
299 Arora 2015). We speculated that the over-composting of CC and C caused the consumption of a
300 large amount of nutrients and led to the relatively poor mushroom yield. Our previous studies
301 found that the size of corn cob and corn straw affected the composting process and mushroom
302 production under the same conditions (Yu et al. 2018). Hence, further investigations are required
303 to elucidate if a more serious structural destruction of AS by the edible fungal pretreatment could
304 lead to higher production.

305 It has been confirmed that *A. bisporus* is a classical example of sustainable food production,
306 which is cultured on compost made from agricultural waste products. The edible fungal compost
307 production is a process involving the bioconversion of raw materials into a composted mixture,
308 of which the temperature increases to 80°C owing to thermophilic microorganisms (Zhang et al.
309 2019). The activity of microorganisms alters the natural decomposition of lignocellulose in the
310 compost of *A. bisporus* (Jurak et al. 2014). The compost preparation (Phase I and II) of a high
311 yielding substrate is probably the most critical phase of mushroom production (Sharma 1991).
312 Composting is an organic materials decomposition process, in whichit has been confirmed that
313 a range of successional taxa microbial convert wheat straw into compost in the thermophilic
314 composting process, which is performed by a microbial organisms consisting of the thermophilic
315 proteobacteria and actinobacteria, and thermophilic fungus (Kertesz and Thai 2018). In the
316 current study, the structures of microflora communities were found to change over the course of
317 the fermentation time, based upon the outcome of the OTUs and dominant phyla and genera
318 observed. It is worth mentioning that the Shannon and Simpson parameters indices showed that

319   samples in Phase I.S possessed the highest bacterial community diversity. Moreover, considering
320   the differences between microflora in composts, both the PCoA and NMDS analysis indicated
321   that the species composition of Phase II samples were different from that of the Phase I samples.

322   Xiao et al. (2018) used high-throughput sequencing to reveal that Proteobacteria was the
323   dominant phylum in thermophilic anaerobic fermentative stages. A previous study reported that
324   Firmicutes, Proteobacteria, Actinobacteria, and Bacteroidetes were the most dominant phyla
325   (Vieira and Pecchia, 2018). Similarly, in this study, the predominant phyla were observed to be
326   Firmicutes, Proteobacteria, and Actinobacteria. The present study also indicated that the relative
327   abundance of the genus *Thermobispora* increased along with fermentation time, while the genera
328   of *Pseudoxanthomonas* and *Thermobifida* decreased. Our previous study(Cao et al. 2018)
329   showed that *Thermobispora*, *Thermopolyspora*, *Ruminiclostridium*, *Thermobacillus,* and
330   *Bacillus* were the predominant genera bacteria in Phase I.T and II in wheat straw consistent with
331   the present study. Studies by both Szekely et al. (2009) and Zhang et al. (2019) supported our
332   data as they found that the genera *Thermopolyspora*, *Pseudoxanthomonas,* and *Thermobifida*
333   were more abundance in mature compost. In addition, it has been reported that the genus
334   *Thermopolyspora,* comprising a cellulolytic actinomycetes species, is highly enriched in mature
335   mushroom compost based on wheat straw (Zhang, et al. 2014). Our previous study also reported
336   that the genera *Thermobispora* and *Thermopolyspora* were predominant in phases I.T and II of
337   wheat straw substrate, by LEfSe and ternary plots (Cao et al. 2018).

Conclusion

339   The results of this study demonstrated that six different AS exhibited differences in the
340   progression of degradation under the same compost condition and the WS, RS, and CS produced
341   a significantly higher mushroom yield by 16 day of composting. The genera *Thermobispora*,
342   *Thermopolyspora,* and *Vulgatibacter*   may play an important role in the different AS
343   degradation. Our findings could be a clue to adjust formulations and compost methods to obtain
344   high-yield mushroom compost cbased on different AS in the future. China is a large agricultural
345   country, so the development of different AS from different areas as substrates for *A. bisporus*
346   cultivation has the potential to reduce the cost of transporting raw materials and increase farming
347   profits.

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473   **Figure legends**

474   **Fig. 1** Temperature change of fermentation chamber .

475   **Fig. 2** Chemical properties of six different AS compost changes in the C/N ratio(A), crude fiber

476   content(B), and the total N amount (C) from the M to Phase II. The red line(—o—) in the figure A

477   represents the changes in C/N ratio of different stages of fermentation. WS: wheat straw

478   substrate; RS: rice straw substrate; CS: cotton straw substrate; CC: corncob substrate; C: corn

479   straw substrate; B: bagasse substrate. The C/N ratio was obtained based on total nitrogen

480   according to the Kjeldahl method and carbon content according to the $K_2Cr_2O_3$ oxidation method.

481   The total N is expressed as a ratio of protein in 100 g dry material.

482   **Fig. 3** Change in the dry matter loss, and contents of cellulose, hemicellulose, and ADL of AS

483   substances between material phase and Phase II.

484   **Fig. 4** TEM images of AS structural changes after composting.A-F represents the microstructure

485   of the original AS material; a-f represents the AS microstructure after Phase II composting: A

486   and B are the CS raw and composting materials, respectively; C and D are WS raw and

487   composting materials, respectively; E and F are the RS raw and composting materials,

488   respectively; G and H are the CC raw and composting materials, respectively; I and J are the C

489   raw and composting materials, respectively; K and L are the raw and composting materials,

490   respectively.

491   **Fig. 5** Summary of the microflora community of compost samples in different phases. A

492   represents OTUs Venn diagram; B and C represent the microflora community of compost at the

493   phylum and genus level; D and E represent the α-diversity parameter (Shannon and Simpson

494   indices); F and G represent PCoA and NMDS plot analysis of the microflora community of

495   compost samples; H represents the comparison of the relative abundance of bacterial strains at

496   the genus level.

497   **Fig. 6** Analysis of microflora community structure differences in the two-stage composts of *A.*

498   *bisporus*. A represents LEfSe analysis for the four treatments. B represents a ternary plot about

499   the I.F, I.S, and I.T treatments; C, D, and E represents the t-test concerning I.F vs. II, I.S vs. II,

500   and I.T vs. II, respectively.

Table 1 Analysis of mushroom yield (*Agaricus bisporus*) cultured on six different AS-based substances

1   **Table 1**   Analysis of mushroom yield (*Agaricus bisporus*) cultured on six different AS-based

2   substances

Item	The average of three-year(Mean±SE)[a]		
	TY (g/kg)	T_3 (g/kg)	BE(%)
Cotton straw substrate(CS)	596.7±14.23[a]	501.2±11.30[a]	84.4±3.35[a]
Wheat straw substrate(WS)	569.9±27.15[ab]	478.6±50.58[a]	83.5±5.12[a]
Rice straw substrate(RS)	553.8±11.25[ab]	481.9±27.29[a]	86.8±3.33[a]
Corncob substrate(CC)	498.0±19.86[b]	379.5±19.63[ab]	76.1±1.80[ab]
Corn straw substrate(C)	370.5±12.56[c]	233.8±6.56[c]	63.7±2.104[b]
Bagasse substrate(B)	318.7±16.25[c]	250.6±17.50[bc]	78.9±2.0[ab]
$F_{(5,17)}$[b]	35.75**	19.36**	6.80**

3   [a]Data were expressed as mean ± standard error (n=3). The data in the same column marked with

4   different small case letters are significantly different(Tukey's HSD, $p<0.05$).[b] *F* value in one-

5   way ANOVA: $p<0.05$ (*) or 0.01 (**).

Figure 1

Figure 1 Temperature change of fermentation chamber
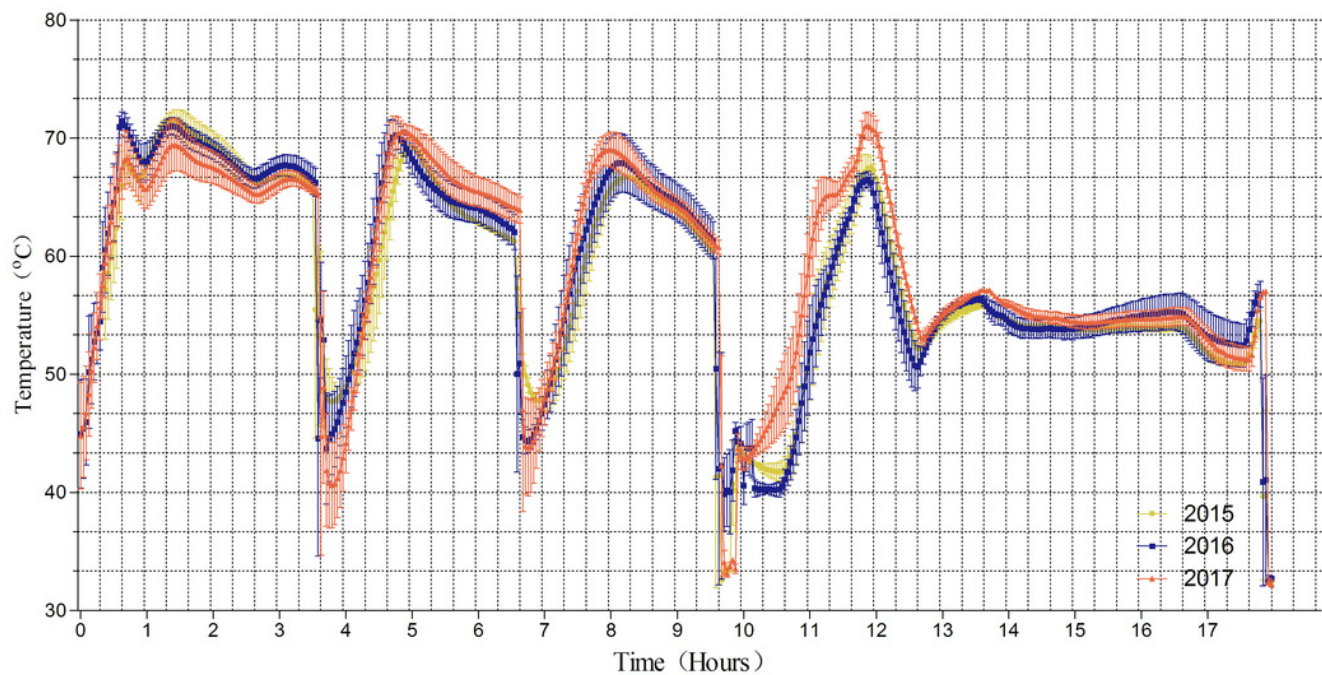
Figure 2

Chemical properties of six different AS compost changes in the C/N ratio(A), crude fiber content(B), and the total N amount (C) from the M to Phase II.

The red line( ) in the figure A represents the changes in C/N ratio of different stages of fermentation. WS: wheat straw substrate; RS: rice straw substrate; CS: cotton straw substrate; CC: corncob substrate; C: corn straw substrate; B: bagasse substrate. The C/N ratio was obtained based on total nitrogen according to the Kjeldahl method and carbon content according to the $K_2Cr_2O_3$ oxidation method. The total N is expressed as a ratio of protein in 100 g dry material.
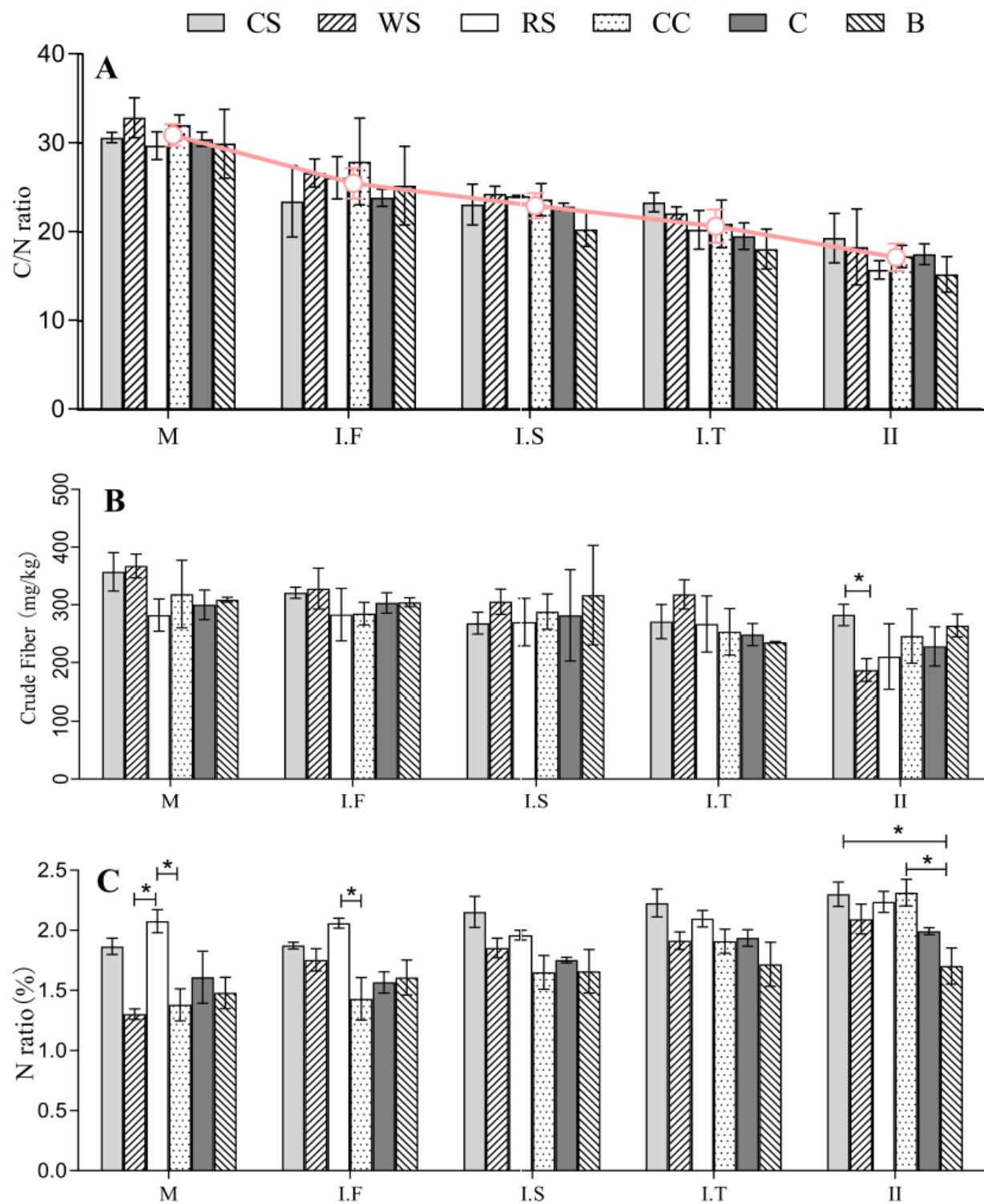
Figure 3

Figure 3 Change in the dry matter loss, and contents of cellulose, hemicellulose, and ADL of AS substances between material phase and Phase II.
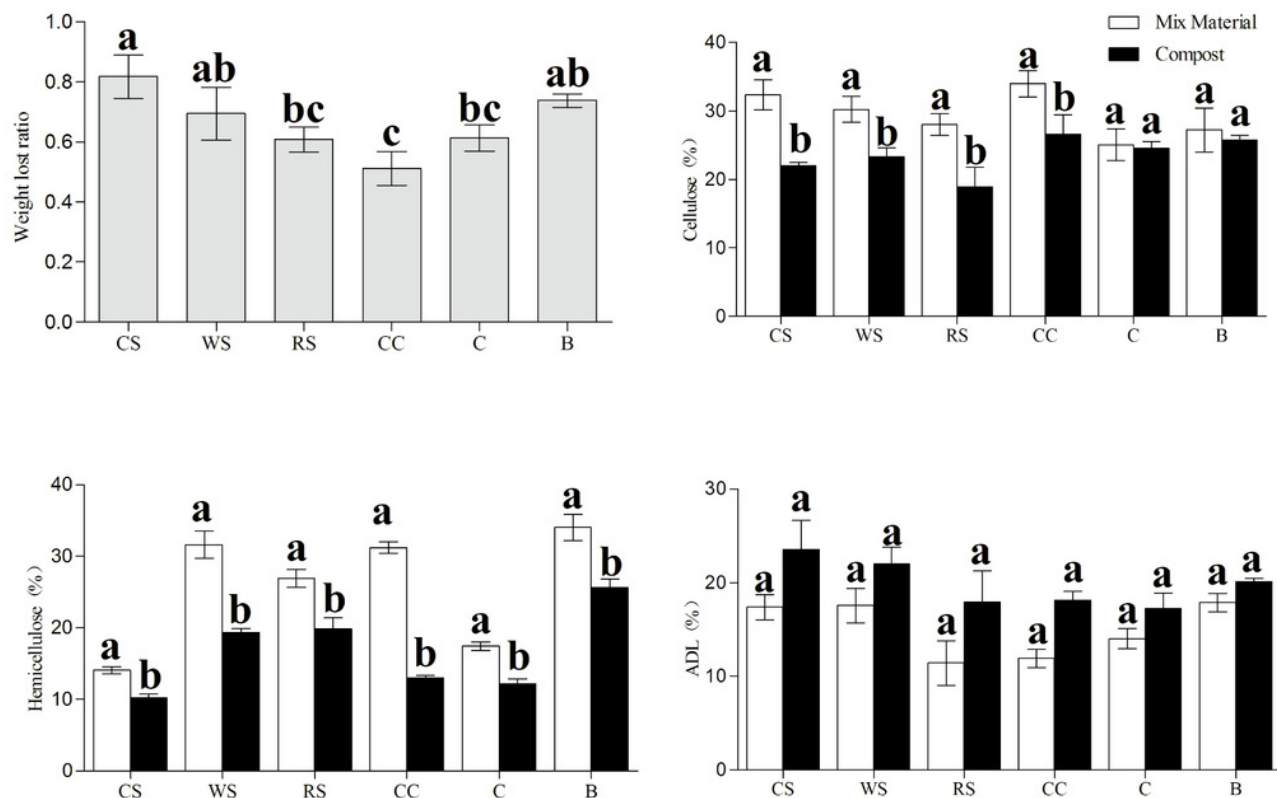
Figure 4

Figure 4 TEM images of AS structural changes after composting

A-F represents the microstructure of the original AS material; a-f represents the AS microstructure after Phase II composting: A and B are the CS raw and composting materials, respectively; C and D are WS raw and composting materials, respectively; E and F are the RS raw and composting materials, respectively; G and H are the CC raw and composting materials, respectively; I and J are the C raw and composting materials, respectively; K and L are the raw and composting materials, respectively.
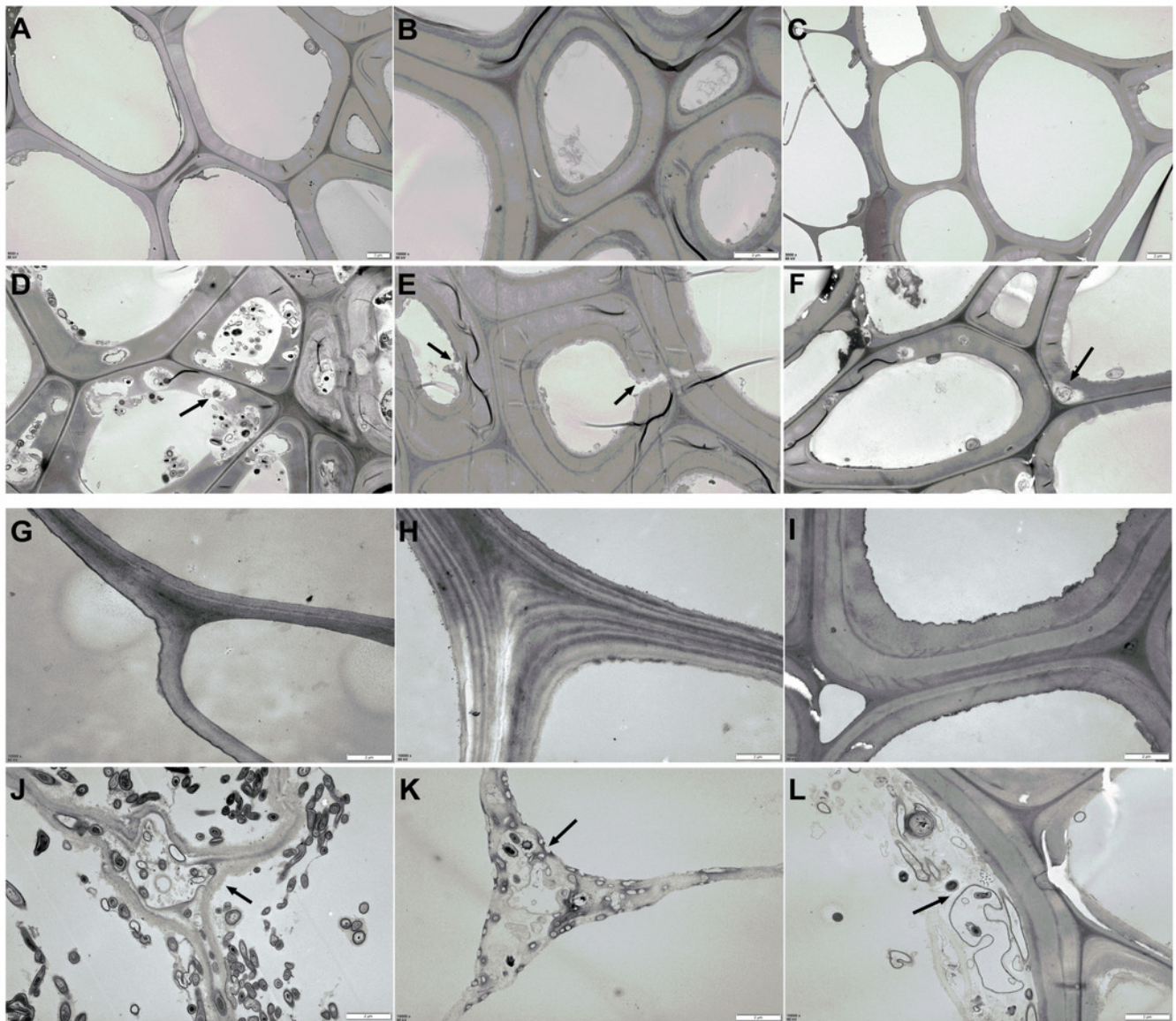
Figure 5

Figure 5 Summary of the microflora community of compost samples in different phases

A represents OTUs Venn diagram; B and C represent the microflora community of compost at the phylum and genus level; D and E represent the α-diversity parameter (Shannon and Simpson indices); F and G represent PCoA and NMDS plot analysis of the microflora community of compost samples; H represents the comparison of the relative abundance of bacterial strains at the genus level.
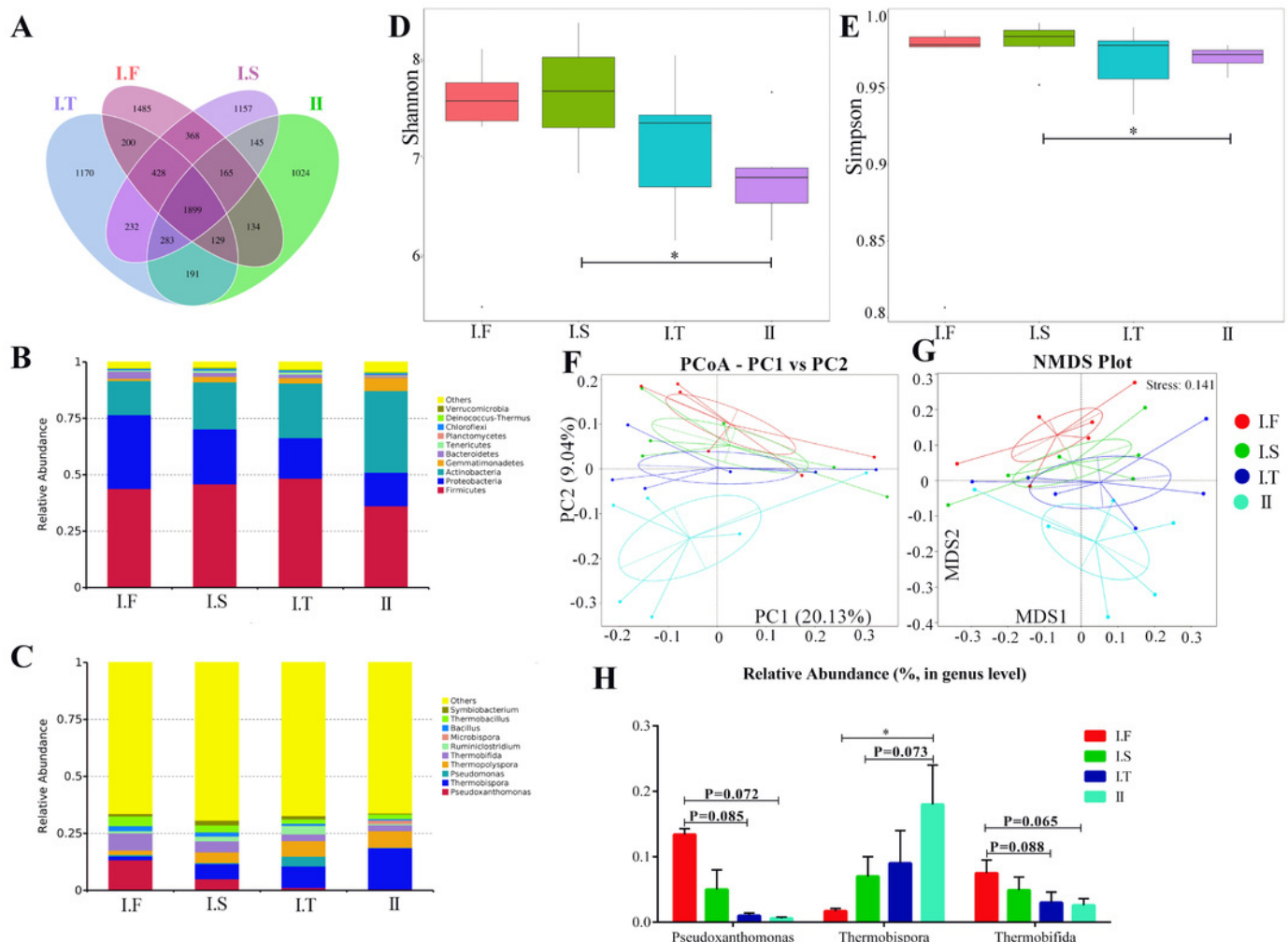
Figure 6

Figure 6 Analysis of microflora community structure differences in the two-stage composts of *A. bisporus*

A represents LEfSe analysis for the four treatments. B represents a ternary plot about the I.F, I.S, and I.T treatments; C, D, and E represents the t-test concerning I.F vs. II, I.S vs. II, and I.T vs. II, respectively.