RESEARCH ARTICLE

2	Rotations improve the diversity of rhizosphere soil bacterial
3	communities, enzyme activities and tomato yield
4	
5	Cui Feng¹¶, Zhengwei Yi¹¶, Wei Qian¹, Huiying Liu¹, Xiaosan Jiang²*
6	
7	¹ Taizhou Institute of Agricultural Sciences, Jiangsu Academy of Agricultural Sciences,
8	Taizhou, China;
9	
10	² College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing,
11	China
12	
13	* Corresponding author
14	Email: gis@njau.edu.cn (XJ)
15	
16	These authors contributed equally to this work
17	

Abstract

18 19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

The use of rotations is an effective strategy to control crop diseases and improve plant health. The soil bacterial communities in the rhizosphere are highly important for maintaining soil productivity. However, the composition and structure of soil bacterial communities in the rotations of vegetable crops remain unclear. In this study, we explored the bacterial diversity and community structure of the tomato rhizosphere, including enzyme activities, yield, and fruit quality, under three different cropping systems: tomato-tomato continuous cropping (TY1), eggplant-tomato (S. melongena) rotation (TY2) and arrowhead-tomato (Sagittaria trifolia-Solanum lycopersicum) rotation (TY3). The composition and diversity of the rhizosphere bacterial communities differed significantly. The diversity was more in the TY2 and TY3 treatments than those in the TY1 treatment. Chujaibacter and Rhodanobacter were two predominant and unique strains detected only in TY1, while the relative abundances of Curvibacter and Luteimonas were the highest in TY2 and TY3, respectively. Moreover, Lysobacterium was a relatively abundant type of biocontrol bacterium found only in the TY3 treatment, which could contribute to alleviating the obstacle of tomato continuous cropping. Compared with the TY1 treatment, the activities of catalase were significantly higher in the TY2 and TY3 treatments. In addition, compared with TY1, the TY2 and TY3 plots increased the following parameters: tomato yields by 24-46%, total soluble solids by 37-93%, total organic acid by 10-15.7% and soluble protein by 10-21%, while the content of nitrate was significantly reduced by 23%. Altogether, compared with the tomato monoculture, the rotations of tomato with eggplant and arrowhead shifted the rhizosphere bacterial communities and improved the yield and quality of the vegetables. Moreover, a tomato rotation, particularly with arrowhead, was an effective way to alleviate the obstacles of continuous cropping.

Keywords: tomato; rotation; bacterial community; enzyme activity; yield

Introduction

43 Tomato (Solanum lycopersicum Mill.) is an important vegetable in solar greenhouses, and it is

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

rich in lycopene, which is antibacterial and has shown potential to be against cancer [1]. In recent years, greenhouse tomato planting has developed rapidly since it not only allows intensive production but also produces high-yield and quality vegetables without seasonal restrictions. However, the area of greenhouse tomato monoculture resulted in soil degradation and salinization [2], which lead to an imbalance of the soil micro-ecosystem, and enrichment of harmful microorganisms [3-4]. Soil-borne pathogens, particularly Fusarium species, cause serious damage to tomato plants, including F. oxysporum f. sp. lycopersici, the causal agent of Fusarium wilt (FW), and F. oxysporum f. sp. radicis-lycopersici, the causal agent of Fusarium crown and root rot (FCRR) [5-7]. Therefore, it is urgent to adopt appropriate soil management measures to alleviate these continuous cropping obstacles. Crop rotation has been identified as an alternative strategy to maintain soil quality and vegetable productivity [8-9]. Previous studies have shown that reasonable crop rotation can improve the soil microecological structure [10-11], increase the number of soil bacteria, alter the community structure [12-13], enhance soil enzyme activity, and promote crop growth, which can effectively reduce continuous cropping obstacles [14,15]. To improve soil microbial diversity by rotation, it may require the use of specific crop combinations, which are expected to have a greater impact on soil microbial diversity [16]. The cultivation of maize (Zea mays L.) in rotation with tomato reduced the populations of nematodes [17], while the cultivation of banana (Musa spp.) in rotation with pineapple (Ananas comosus) reduced the amount of FW pathogens in the soil fungal community [18]. These examples highlight the importance of crop rotations in the suppression of soilborne diseases. One of the inhibitory mechanisms is related to changes in the abundances of beneficial and pathogenic soil bacteria [19].

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

Soil microbes influence crop productivity, nutrient uptake and agricultural sustainability and play important roles in nutrient cycling [20,21]. Certain species of soil microbes can protect plants from soilborne pathogens and improve plant growth by acting as biocontrol agents (BCAs) [22]. For example, the fungal genus *Mortierella* appears to enhance the suppression of FW disease in vanilla (Vanilla planifolia) [23]. Members of the bacterial genera Pseudomonas and Bacillus have been verified as two representatives for effective BCAs against different fungal pathogens, including the tomato FW pathogen [24]. In recent years, the interaction between rhizosphere organisms has become the subject of increasing amounts of researches [25]. The rotation of tomato with xerophytes or rice have been assessed by many studies, but there are few studies on rotation with aquatic vegetables. Arrowhead (Sagittaria sagittifolia) is an exceptional aquatic vegetable in the Lixiahe region, which is rich in starch, protein, vitamins and other nutrients. Arrowhead is a water-loving plant, so it is a good choice for crop rotation. However, there are few studies on the cultivation of S. sagittifolia and tomato, particularly on the effects of crop rotation on soil microbial and physicochemical properties. In addition, the effect of this type of crop rotation on bacteria is unclear. Soil enzymes increase the rate at which plant residues decompose and release their available nutrients [26,27]. Soil enzyme activity is the cumulative effect of long-term microbial activity and the activity of viable populations at sampling [28]. It plays an important role in the soil ecosystem, including the cycling of carbon (C), nitrogen (N), phosphorus (P) and plant nutrient transformations, which provide information on the ability of soils to perform biogeochemical reactions [29]. Furthermore, many studies have illustrated that human activities, such as field management measures, can significantly affect the activities of soil enzymes

[30,31]. Different farming methods may directly or indirectly affect soil enzyme activities, including the soil physicochemical properties, vegetation types, plant roots, and soil microorganisms [32,33]. Therefore, the soil enzyme activities affected by rotation can be used to describe the changes in soil quality owing to continuous cropping to better understand the function and quality of soil ecosystems. In this study, we evaluated the effects of different vegetable rotations (monoculture of tomato and rotations of eggplant [Solanum melongena]-tomato and arrowhead [Sagittaria sagittifolia]tomato) on soil bacterial communities, and evaluated the most effective tomato rotation system. 16S rRNA gene was used to estimate the microbial community structures. The soil enzyme activity, tomato yield and fruit quality were measured simultaneously. We aimed to (i) examine the response of soil bacterial communities to varied tomato rotations and identify significant differences in the composition of taxa, (ii) evaluate the variation in soil enzyme activity under these three rotations, and (iii) compare the changes in tomato yield and quality. This study is the first to analyze the effects of arrowhead-tomato rotation on tomato growth and rhizosphere soil environment, and will provide insights into the effectiveness of tomato rotation systems in alleviating the productivity obstacles caused by tomato monoculture.

Materials and Methods

Study site description

88

89

90

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

A plastic greenhouse experiment was conducted at an experimental plot of the Taizhou Academy of Agricultural Sciences, Taizhou, Jiangsu Province, China (32°54′N, 11°99′N). The greenhouse monoculture of tomato had been used in this plot for more than two years before this experiment was established. The area is a subtropical monsoon climate, and the annual

mean temperature, annual mean accumulated temperature and sunshine duration were 14.5° C, $5,365.6^{\circ}$ C and 2,205.9 h, respectively. The annual mean precipitation was 991.7 mm, and there were 117 annual mean rainy days. The USDA classification system identified the soil as loamy. The surface soil (0–20 cm) in the greenhouse had a pH of $7.8, 25.8 \text{ g} \cdot \text{kg}^{-1}$ organic matter, $1.68 \text{ g} \cdot \text{kg}^{-1}$ total N (TN), $16.5 \text{ mg} \cdot \text{kg}^{-1}$ available P (AP), and $211 \text{ mg} \cdot \text{kg}^{-1}$ available K (AK) before the experiment was initiated.

Experimental design

The field experiment was conducted from July 2018 to July 2020, and two crops of tomatoes were planted from July 2018 to July 2019. The continuous (round) planting period of the experimental vegetables was from August 2019 to January 2020, and the growing period of experimental tomatoes was from March 2020 to July 2020. Three vegetable rotations, consisting of monoculture tomato (Japanese Fenwang, TY1), eggplant (Suqi 3)-tomato rotation (TY2), and arrowhead-tomato rotation (TY3), were arranged in a randomized block design with three replicates. Each plot within the plastic greenhouse was 12 m × 1.2 m. There were 48 tomato plants on each plot, and the distance between the two plants was 50 cm. A 0.5 m wide buffer row separated the adjacent plots. In addition, the buffer line was wrapped with black plastic film to avoid mutual interference between the plots. The field management options, including transplantation, irrigation and the use of pesticides, were consistent with the local cultivation standards. Among them, arrowhead was grown in moist culture.

Soil sampling

Five rhizosphere soil subsamples were randomly collected from each replicate plot and mixed

as a composite sample for analysis at the tomato harvest stage. The collection of rhizosphere soil involved firstly removing approximately 3 cm of the surface soil. The entire tomato root was taken out, and the rhizosphere soil was separated from the bulk soil by gently shaking. All the soil samples were screened via a 2 mm sieve and thoroughly homogenized after all the visible roots and impurities had been removed. One portion of the homogenized fresh soil sample was stored at -70°C for DNA extraction, and the other one was used immediately to determine the soil enzyme activity.

Measurements and methods

131

132

133

134

135

136

137

138

139

140

141

142

143

144

145

146

147

148

149

150

151

Extraction and Sequence of Soil Microbial DNA

The soil DNA was extracted using a Fast DNA Spin Kit for Soil (MP Biomedicals, Cleveland, OH) according to the manufacturer's instructions. The quality of DNA was detected by 0.8% agarose gel electrophoresis and quantitatively analyzed by NanoDrop ND-1000 (Thermo Fisher Scientific, Waltham, USA). MA, The barcoded primers 16S5/16S2R (5'-GGAAGTAAAAGTCGTAACAAGG - 3'/5'- CTGCGTTCTTCATCGATGC -3') were used to amplify 2 μL of DNA template, 0.25 μL of Q5 DNA polymerase, and 8.75 μL of ddH₂O. The cycling program was 98°C for 2 min; 27 cycles of 98°C for 15 s, 55°C for 30 s, 72°C for 30 s; and 72°C for 5 min. The PCR amplification products were detected by 2% agarose gel electrophoresis, and the target fragments were recovered by cutting the gel and using a gel recovery kit from Axygen (Axygen Biosciences, Union City, CA, USA). The amplicon libraries were pooled and sequenced using a 2 × 250 base paired Illumina MiSeq platform (Illumina, Inc, San Diego, CA, USA) and then stored at 10°C.

Soil enzyme assays

152

153

154

155

156

157

158

159

160

161

162

163

164

165

166

167

168

169

170

171

172

173

The activities of urease, catalase, sucrase and alkaline phosphatase were determined as described by Hu et al. (2014) [34] with some modifications. The urease activity was analyzed using the phenol-sodium hypochlorite colorimetric method. In brief, 10 mL of 10% urea solution and 20 mL of citrate buffer (pH 6.7) were added to a 50 mL conical flask that contained 5 g of soil. After 24 h of incubation at 37°C, the suspension was filtered, and then 0.5 mL of the filtrate was added to a 50 mL flask that contained 20 mL of distilled water and 4 mL of sodium phenol solution. Next, 4 mL of sodium hypochlorite solution was added to the flask and mixed. The volume was adjusted to 50 mL with distilled water, and the absorbance was monitored at 578 nm. The catalase activity was measured using the potassium permanganate (KMnO₄) titration method. Briefly, 5 mL of 0.5% H₂O₂ was added to 1 g of soil for 20 minutes, and the residual H₂O₂ in the soil was titrated with 0.01 M KMnO₄. The H₂O₂ consumed was expressed as catalase activity. The 3,5-dinitrosalicylic acid colorimetric method was used to analyze the soil sucrase activity. A volume of 0.2 mL of toluene and 5 mL of N-acetylmuramic acid (MUB) were added to a 50 mL Erlenmeyer flask that contained 3 g of soil and they were shaken for a few seconds to mix the sample. Next, 5 M of 10% sucrose was added and shaken again. The flask was sealed, and the soil sample was incubated at 37°C for 24 h. A volume of 1 mL of filtrate, 2 mL of 2 M NaOH, 2 mL of the color reagent and 5 mL of deionized water were added to a 50 mL test tube and flushed with N_2 for 10 min. The tubes were all placed in a boiling water bath for 5 min with inverted 50 mL beakers on their tops and then cooled to room temperature. A spectrophotometer was used to determine the color intensity at a wavelength of 540 nm. The soil phosphatase activity was determined using the disodium phenyl phosphate

method. In short, 5 g of soil sample was added to a 250 mL flask, and 2 mL of toluene was used to inhibit the growth of microorganisms. After standing for 15 min, 20 mL of 0.5 % (w/v) disodium phenyl phosphate prepared in acetic acid buffer (pH 5) was added to the flask and then incubated at 37°C for 24 h. Subsequently, after adding 100 mL of 0.3% Al₂(SO₄)₃ to the flask, the soil sample was filtered, and 3 mL of the filtrate was added to a 50 mL flask. A volume of 5 mL of borate buffer (pH 9.4) and four drops of indicator were added. The volume was adjusted to 50 mL, the absorbance at 660 nm was determined. **Determination of vegetable quality** The content of VC was determined by 2, 6-dichloroindigophenol titration [35]. The content of TSS was determined by refractometry [36]. The content of titratable acid was determined by acid-base titration [37]. The nitrate content was determined by UV spectrophotometry [38]. The soluble protein content was determined with Coomassie brilliant blue using bovine serum albumin as the standard [39]. The soluble total sugar content was determined by the anthrone method [39]. Data analysis PRINSEQ, PANDAseq, USEARCH, and self-developed Perl were used to analyze the microbial diversity of sequencing results. The data of tomato yield and quality were processed using Microsoft Excel 2010 (Redmond, WA, USA). SPSS 18.0 (SPSS, Inc., Chicago, IL, USA) was used for statistical analyses.

Results

174

175

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

194

Soil bacterial community diversity among tomato planting patterns

All the samples were rarefied to the same number of reads as the sample with the lowest reads, which was 168,816. The bacterial sequences were clustered at 97% similarity and annotated in the RDP database to obtain 2,673, 3,074, and 3,266 bacterial operational taxonomic units (OTU) for TY1, TY2, and TY3, respectively (Table 1). The coverage rates of the 16S rRNA sequencing in the three soil samples were 0.9992, 0.9995, and 0.9996, respectively, indicating that the sequencing depth had basically covered all the species in the sample.

The diversity analysis indicated that TY1 (802) had the lowest Chao1 index, while TY3 (1,065) had the highest Chao1 index, and the Chao1 index of TY2 (996) was between those of TY1 and TY3. From TY1 to TY3, the Shannon index gradually increased (5.1, 6.7, and 7.1, respectively). It is apparent that the diversity and richness of tomato soil bacteria after rotation were higher than those of continuous cropping, and the diversity and richness of soil bacteria were the highest under the mode of arrowhead-tomato rotation. The bacterial diversity and richness decreased under continuous cropping, and the rotation could increase the microbial species in soil.

Table 1. Soil bacterial diversity indices of different planting patterns.

Treatment	Sequence numbers	OTU numbers	Chao1 index	Shannon index	Simpson index	goods coverage
TY1 ¹	168816	2,673	802	5.1	0.92	0.9992
TY2 ²	168816	3,074	996	6.7	0.97	0.9995
TY3 ³	168816	3,266	1065	7.1	0.98	0.9996

¹ Tomato continuous cropping; ² Eggplant-tomato rotation; ³ Arrowhead-tomato rotation.

The Venn diagram intuitively showed the number of common and unique OTUs of different samples (Fig 1). TY1, TY2, and TY3 contained 2,673, 3,074 and 3,266 OTUs, respectively, with 2,125 OTUs shared by the three soil samples. Thus, more than 79% of the OTUs were the

same in each soil. The number of specific bacterial OTUs was 142, 221, and 465, respectively. TY3 had the highest number of specific bacterial OTUs, which was 3.27-fold higher than that of TY1, indicating that more specific bacterial groups existed in the arrowhead-tomato rotation.

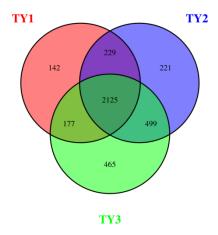


Fig 1. Venn diagram showing the distribution of OTUs in different soil samples. TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

Comparison of the Soil Bacterial in Different Soil Samples at the Level of Genera

The genera composition of soil bacteria showed that there were at least 769 bacterial genera in all the soil bacterial communities, and the first 21 genera based on relative abundance were selected for comparison. In the identified bacterial community, *Chujaibacter* and *Rhodanobacter* were the two predominant and unique strains detected only in TY1, while no such bacteria were found in the other two crop rotation patterns (Fig 2). Some studies have shown that species of *Rhodanobacter* positively correlated with the incidence of FW [40]. An analysis based on OTUs within the species abundance information was visualized at the genus level with the five most abundant genera. The abundance of soil bacterial genera was TY1>TY2>TY3. TY1 had the highest relative abundance of *Chujaibacter*, while TY2 had the highest abundance of *Curvibacter* (Fig 3). *Luteimonas* was the most abundant in TY3, and

Lysobacter was only found in TY3. These results indicated that there were differences in the species, diversity, and richness of soil bacteria among different cropping patterns, and the rotation helped to enhance the numbers of beneficial soil bacteria.

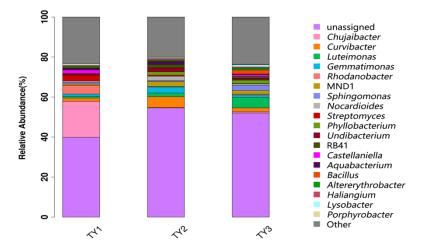


Fig 2. Comparison of bacterial classification at the genus level in soil of different planting patterns. TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

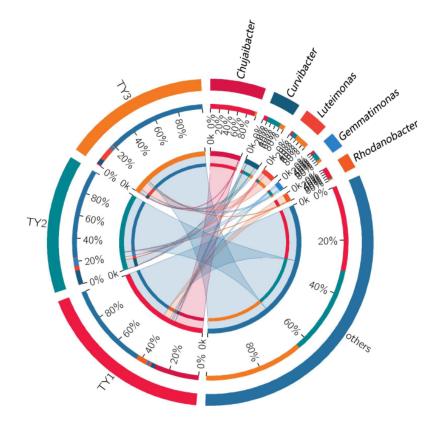


Fig 3. Profiling circles of the top five species in the different sample genera classification levels. TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation. In the graph of collinearity between samples and species, the left semicircle represents the species abundance composition of samples, and the right semicircle represents the distribution proportion of species in different samples under this clustering level.

3.3. Distribution curves of soil microbial abundance among different planting modes

The richness and evenness of species and the TY3 treatment curve is relatively flat (Fig 4), indicating that the soil bacterial composition is the highest uniformity under the irrigated and upland rotation mode. The curve width of TY3 and TY2 treatment was significantly wider than TY1, indicating that the soil bacterial species diversity and richness of TY3 and TY2 were higher.

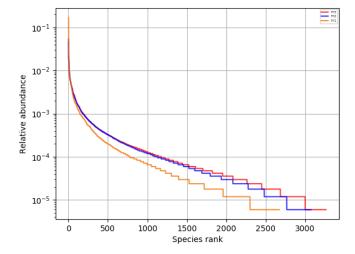


Fig 4. Abundance distribution curve for three samples. The horizontal axis shows the relative abundance of OTU in descending order, while the vertical axis shows the proportion of relative abundance of OTU. TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

Effects of different planting patterns on soil enzyme activities

The activity of urease differed significantly among the three planting patterns, and the amount of activity was as follows: TY1>TY2 >TY3 (Table 2). There were significant differences in catalase activity among the three cropping modes (P<0.05). TY3 had the highest level of activity. In general, soil that was more fertile had higher amounts of sucrase activity. The sucrase activities of the three samples were as follows: TY3>TY1>TY2, indicating that the arrowhead-tomato rotation could increase soil fertility. Phosphatase can be divided into three types: acid phosphatase, neutral phosphatase and alkaline phosphatase. The soil samples collected in this experiment were all alkaline soil samples with pH values > 7, so only alkaline phosphatase activity was measured. The results of the three soil samples show that the amount of alkaline phosphatase activity in TY1, TY2 and TY3 differed significantly (P<0.05). TY2 had the highest activity of alkaline phosphatase, while there was no significant difference with TY1 (P<0.05). This is probably due to that tomato and eggplant are solanaceous. The change in enzyme activity could be caused by continuously cropping tomato, which resulted in root secretions that altered the quantity of soil organic matter and caused changes to the microflora.

Table 2. Effects of different planting patterns on the enzyme activities of soil.

Treatment	urease	catalase	sucrase	alkaline
Treatment	urease	Catalase	sucrase	phosphatase
TY1	0.51 a ¹	2.07 c	377.01 b	2.54 a
TY2	0.46 b	2.19 b	370.16 b	2.60 a
TY3	0.31 c	3.15 a	515.76 a	2.00 b

¹ Different lowercase letters in the same column indicate significant differences (P < 0.05). TY1,

255

256

257

258

259

260

261

262

263

264

265

266

267

268

269

Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

Effects of different planting patterns on the yield and quality of tomato

There were significant differences in tomato yield among the three cultivation patterns (Fig 5). Compared with continuous cropping, the eggplant-tomato and arrowhead-tomato rotations increased the yield of tomato by 28.6% and 16.3%, respectively, indicating that rotating the crops helped to increase the yields of tomato.

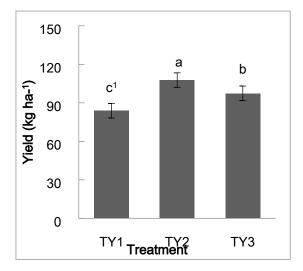


Fig 5. Comparison of tomato yields between different planting patterns.

¹ Different lowercase letters in the same column indicate significant differences (*P* < 0.05). TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

There were some differences in tomato quality under the three different cultivation patterns (Fig 6). In general, the contents of TSS, VC, soluble proteins and soluble total sugars in the TY2 tomatoes were all higher than those in TY1, indicating that crop rotation helped to improve fruit quality. The nitrate content of tomato under the different cultivation patterns differed, and

the difference between an irrigation-upland rotation and continuous cropping was highly

significant (P<0.05), and TY3 was the lowest, indicating that an irrigation-upland rotation

could reduce the nitrate content of tomato fruit.

289

290

291

292

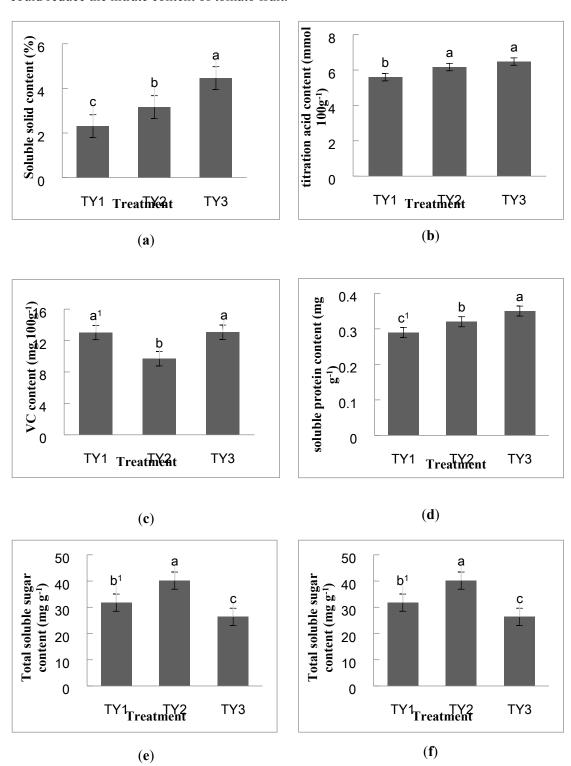


Fig 6. Comparison of tomato quality among different planting patterns.

¹ Different lowercase letters in the same column indicate significant differences (P < 0.05)

TY1, Tomato continuous cropping; TY2, Eggplant-tomato rotation; TY3, Arrowhead-tomato rotation.

Discussion

293

294

295

296

297

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

Bacteria are the most widely distributed and abundant components of soil microorganisms [41], accounting for 70%-90% of the total number of soil microorganisms. This has resulted in their use as one of the most sensitive indicators for changes in soil nutrients [42]. This study used high-throughput sequencing technology to compare the differences in the community structure and abundance of soil bacterial species under different tomato planting patterns. The soil bacterial community structure and abundance differed somewhat between the rotation patterns and continuous cropping. Continuous cropping can lead to an imbalance in the microbial population structure in the soil and a decrease in soil enzyme activity. Crop rotation can improve the microbial diversity, increase the abundance of soil bacteria, prevent the occurrence of soilborne diseases, and increase the crop yields [43]. The incidence of bacterial wilt, blight and root-knot nematode disease in arrowhead-tomato rotation fields were 12%, 16% and 8% lower than those in conventional fields, respectively [44]. The number of bacterial OTUs in the TY1, TY2 and TY3 samples were 2,673, 3,074 and 3,266, respectively. The Chao1 and Shannon indices ranked TY3>TY2>TY1. This is consistent with the results of Liu et al. (2021) [45] who found that the abundance of bacteria in tomato continuous cropping and rotation soil differed substantially. The Venn analysis showed that the number of unique OTUs in TY3 and TY2 were greater than those in TY1. A clustering analysis showed that the three soil samples had different dominant genera. Rhodanobacter was the dominant genus in TY1 and positively correlated with the incidence of FW. Moreover, the rotation system had more beneficial and

316

317

318

319

320

321

322

323

324

325

326

327

328

329

330

331

332

333

334

335

336

diversity bacterial species. Aquabacterium, Luteimonas and Gemmatimonas were found in TY2 and TY3, and some metabolites of these bacteria are antimicrobial [45-46]. Gemmatimonas can prevent disease, act as an insecticide, promote growth, remediate heavy metal pollution, and decompose toxic gases [45]. Lysobacter is a unique strain of TY3, which can resist adverse environments and prevent soil diseases [47]. Therefore, tomato continuous cropping can lead to the accumulation of harmful bacteria in the soil, while crop rotations can change the bacterial structure in the soil, which help to increase the numbers of biocontrol bacteria and other types of beneficial bacterial species. This was particularly true for the arrowhead-tomato rotation soil, which was much more likely to contribute to the control of microbial diseases. This can be explained by the increase in soil microorganisms of some types of beneficial bacteria that thrive under rotation cultivation and are benefit for the crop growth. The distribution curves of the soil microbial abundance showed that the diversity and evenness of soil bacterial species and the abundance of each species under rotation were higher than those under continuous cropping, and the evenness of soil bacterial composition was higher under arrowhead-tomato rotation. This indicates that crop rotation can increase the number and activity of soil microorganisms, which is consistent with a study by Wang et al. (2002) [48]. Soil enzymes are the most active compounds in the soil and important indicators that reflect biochemical processes in the soil [49], so they play an important role in the material cycle and energy transformation of the soil ecosystem. Studies have shown that years of facility planting and cultivation methods have important effects on soil microorganisms and enzyme activities [42,50]. Wu et al. (2000) [51] found that the activities of catalase, urease and sucrase in the rotation soil were significantly higher than those in continuous cropping soil. Urease is the main

338

339

340

341

342

343

344

345

346

347

348

349

350

351

352

353

354

355

356

357

358

source of available nitrogen in soil, which is conducive to the conversion of organic nitrogen to ammonium nitrogen for plant growth and metabolism [52], and the activity of urease is an important indicator of the presence of nitrogen fertilizer. Catalase can destroy the hydrogen peroxide generated by biochemical reactions in the soil and reduce its deleterious effects on plants [52]. The strength of sucrase activity reflects the degree of soil maturation and fertility, and plays an important role in increasing the amount of nutrients in the soil [40]. Phosphatase can hydrolyze soil organophosphorus compounds and release corresponding alcohols and inorganic phosphorus, and its activity is also one of the important indicators of soil fertility (particularly phosphate fertilizer) [40]. In this sense some enzyme activities are not apparent. Further study will be needed. The yield of tomato is an important index that reflects its economic value, and this study showed that crop rotation helped to improve the yields of tomato. Compared with continuous cropping, the yield of tomato increased by 28.6% and 16.3% in the eggplant-tomato and arrowhead-tomato rotations, respectively. Crude protein, VC and reducing sugar contents are important indicators that reflect the nutritional quality of vegetables, which affect their commodity value. Our study showed that there were some differences in tomato quality under three different cultivation patterns. In general, the contents of TSS, total organic acids, VC and soluble proteins in tomato under rotation were higher than those under continuous cropping. The nitrate contents of the eggplant-tomato and arrowhead-tomato rotations were 5.8% and 27.5% lower than that of continuous cropping, respectively. Nitrate is an important quality indicator of vegetables. The activity of catalase was the lowest in TY1, which could be related to the formation of tomato continuous cropping obstacles. The activity of catalase was the

highest in TY3, which indicated that the ability of an irrigation-drought rotation mode was stronger and better to be able to cleanse the soil, and it was one of the better cultivation methods to avoid the continuous cropping obstacles of tomato. Sucrase was the most active in TY3, and alkaline phosphatase was the most active in TY2, indicating that rotations are better at maintaining soil fertility. However, urease and phosphatase were the least active in TY3, indicating that crop rotation could affect the soil N and P cycles and promote the utilization of nutrients. In general, the activities of soil enzymes in the three soil samples did not show a strong trend, which could be owing to the short continuous cropping period of tomato in this study. Another factor is that tomato plants are solanaceous, so the difference can cause human cyanosis and even induce cancers of the digestive system [53]. Kang et al. (2016) [54] showed that in non-organic planting systems, vegetables with a higher content of nitrate have a lower content of VC. Dai et al. (2017) [55] found that improving the soil microbial community abundance and diversity can enhance disease resistance in the soil and increase the amounts of nutrients [55], which is consistent with the results of our study. Therefore, crop rotation can improve the yield and quality of tomato and reduce the nitrate content.

Conclusion

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

375

376

377

378

379

380

Compared with tomato continuous cropping, the tomato rotation patterns significantly improved the abundance of beneficial bacteria. Moreover, the rotations increased the activities of soil enzymes, such as catalase, sucrase, and alkaline phosphatase. The rotations also improved the quality of fruit with an increase in the content of total soluble solids, soluble proteins, and VC, while it decreased the content of nitrate nitrogen. Thereby, the tomato rotation patterns improved the yield of tomato. It is worth noting that in the two types of rotation,

arrowhead-tomato rotation was more effective at relieving the obstacles of continuous cropping.

This study was the first to verify the effects of crop rotation of arrowhead and tomato on soil microbial and physicochemical properties. In practical production, the arrowhead-tomato rotation is recommended to promote crop growth.

Author Contributions

Methodology: Cui Feng.

Formal analysis: Cui Feng and Zhengwei Yi.

Investigation: Wei Qian and Huiying Liu.

Writing—original draft: Cui Feng.

Writing—review and editing: Cui Feng and Xiaosan Jiang.

All authors have read and agreed to the published version of the manuscript.

References

- 393 1. Suwanaruang T. Analyzing lycopene content in fruits. Agriculture and Agricultural Science
- 394 Procedia. 2016; 11: 46-48. https://doi.org/10.1016/j.aaspro.2016.12.008.
- Liu T, Cheng Z, Meng H, Ahmad I, Zhao H. Growth, yield and quality of spring tomato and
- physicochemical properties of medium in a tomato/garlic intercropping system under plastic
- tunnel organic medium cultivation. Scientia Horticulturae. 2014; 170: 159-168.
- 398 https://doi.org/10.1016/j.scienta.2014.02.039.
- 399 3. Li J, Ren G, Jia Z, Dong Y. Composition and activity of rhizosphere microbial communities
- 400 associated with healthy and diseased greenhouse tomatoes. Plant and Soil. 2014; 380: 337-347.
- 401 https://doi.org/10.1007/s11104-014-2097-6.
- 402 4. Fu H, Zhang G, Zhang F, Sun Z, Geng G, Li T. Effects of continuous tomato monoculture on
- 403 soil microbial properties and enzyme activities in a solar greenhouse. Sustainability. 2017; 9:
- 404 317. https://doi.org/doi:10.3390/su9020317.
- 405 5. Butler D. Fungus threatens top banana. Nature. 2013; 504: 195-196.
- 406 https://10.1038/504195a.PMID: 24336262
- 407 6. Chellemi D5O, Wu T, Graham JH, Church G. Biological impact of divergent and management
- practices on tomato crop health. Phytopathology. 2012; 102: 597-608.
- 409 https://doi.org/10.1094/PHYTO-08-11-0219.PMID: 22352308
- Chellemi DO, Rosskopf EN, Kokalis-Burelle N. The effect of transitional organic production
- 411 practices on soilborne pests of tomato in a simulated microplot study. Phytopathology, 2013;
- 412 103: 792-801. https://10.1094/phyto-09-12-0243-r.PMID: 23837543

413	8.	Jiang Y, Liang Y, Li C, Wang F, Sui Y, Suvannang N, et al. Crop rotations alter bacterial and
414		fungal diversity in paddy soils across East Asia. Soil Biology and Biochemistry. 2016; 95: 250-
415		261. https://doi.org/10.1016/j.soilbio.2016.01.007.
416	9.	Soman C, Li D, Wander MM, Kent AD. Long-term fertilizer and crop-rotation treatments
417		differentially affect soil bacterial community structure. Plant and Soil. 2017; 413: 145-159.
418		https://doi.org/10.1007/s11104-016-3083-y.
419	10.	Kurle JE, Grau CR, Oplinger ES, Mengistu A. Tillage, crop sequence, and cultivar effects on
420		sclerotinia stem rot incidence and yield in soybean. Agronomy Journal. 2001; 93: 973-982.
421		https://doi.org/10.2134/agronj2001.935973x.
422	11.	Ai C, Zhang S, Zhang X, Guo D, Zhou W, Huang SM. Distinct responses of soil bacterial and
423		fungal communities to changes in fertilization regime and crop rotation. Geoderma. 2018; 319:
424		156-166. https://doi.org/10.1016/j.geoderma.2018.01.010.
425	12.	Chenu C, Angers DA, Barré P, Derrien D, Arrouays D, Balesdent, J. Increasing organic stocks
426		in agricultural soils: Knowledge gaps and potential innovations. Soil and Tillage Research.
427		2019; 188: 41-52. https://doi.org/10.1016/j.still.2018.04.011.
428	13.	Maarastawi SA, Frindte K, Linnartz M, Knief C. Crop rotation and straw application impact
429		microbial communities in Italian and Philippine soils and the rhizosphere of Zea mays. In Front
430		Microbiol. 2018; 9: 1295.PMID: 29963033
431	14.	Lei Y, Xiao Y, Li L, Jiang C, Zu C, Li T, et al. Impact of tillage practices on soil bacterial
432		diversity and composition under the tobacco-rice rotation in China. Journal of Microbiology
433		2017, 55: 349-356. https://doi.org/10.1007/s12275-017-6242-9.PMID: 28251545

434 Navarro-Noya YE, Gómez-Acata S, Montoya-Ciriaco N, Rojas-Valdez A, Suárez-Arriaga MC, 15. 435 Valenzuela-Encinas C, et al. Relative impacts of tillage, residue management and crop-rotation 436 on soil bacterial communities in a semi-arid agroecosystem. Soil Biology and Biochemistry. 2013; 65: 86-95. https://doi.org/10.1016/j.soilbio.2013.05.009. 437 438 16. Lyu J, Jin L, Jin N, Xie J, Xiao X, Hu L, et al. Effects of different vegetable rotations on fungal 439 community structure in continuous tomato cropping matrix in greenhouse. Frontiers in 440 Microbiology. 2020; 11: 829. https://doi.org/10.3389/fmicb.2020.00829.PMID: 32508762 441 17. Luambano ND, Narla RD, Wanjohi WJ, Kimenju JW, Kerry BR. Integrated management of 442 root-knot nematodes in a tomato-maize crop system using the biocontrol fungus Pochonia 443 clamydosporia. Crop Protection. 2015; 71, 45-50. https://doi.org/10.1016/j.cropro.2015.1-13. 444 18. Wang B, Li R, Ruan Y, Ou Y, Zhao Y, Shen Q. Pineapple-banana rotation reduced the amount 445 of Fusarium oxysporum more than maize-banana rotation mainly through modulating fungal 446 communities. Soil **Biology** Biochemistry. 2015; 77-86. and 86 447 https://doi.org/10.1016/j.soilbio.2015.02.021. 448 19. D'Acunto L, Andrade JF, Poggio SL, Semmartin M. Diversifying crop rotation increased 449 metabolic soil diversity and activity of the microbial community. Agriculture, Ecosystems 450 &Environment. 2018; 257: 159–164. https://doi.org/10.1016/j.agee.2018.02.011. 451 Lychuk TE, Moulin AP, Lemke RL, Izaurralde RC, Johnson EN, Olfert OO, et al. Climate change, 20. 452 agricultural inputs, cropping diversity, and environment affect soil carbon and respiration: A 453 2019; 337: case study in Saskatchewan, Canada. Geoderma. 664-678. 454 https://doi.org/10.1016/j.geoderma.2018.10.010.

455	21.	Armada E, Leite MFA, Medina A, Azcón R, Kuramae EE. Native bacteria promote plant growth
456		under drought stress condition without impacting the rhizomicrobiome. FEMS Microbiology
457		Ecology. 2018; 94: fiy092. https://10.1093/femsec/fiy092.PMID: 29771325
458	22.	Shi L, Du N, Shu S, Sun J, Li S, Guo S. Paenibacillus polymyxa NSY50 suppresses Fusarium
459		wilt in cucumbers by regulating the rhizospheric microbial community. Scientific reports. 2017;
460		7: 41234. https://doi.org/10.1038/srep41234.PMID: 28198807
461	23.	Xiong W, Li R, Ren Y, Liu C, Zhao Q, Wu H, et al. Distinct roles for soil fungal and bacterial
462		communities associated with the suppression of vanilla Fusarium wilt disease. Soil Biology and
463		Biochemistry. 2017; 107: 198-207. https://doi.org/10.1016/j.soilbio.2017.01.010.
464	24.	Aydi Ben Abdallah R, Mokni-Tlili S, Nefzi A, Jabnoun-Khiareddine H, Daami-Remadi M.
465		Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria
466		isolated from Nicotiana glauca organs. Biological Control. 2016; 97: 80-88.
467		https://doi.org/10.1016/j.biocontrol.2016.03.005.
468	25.	Marcel GA, Heijden VD, Martin, Hartmann. Networking in the Plant Microbiome. PLoS
469		Biology. 2016; https://doi.org/10.1371/journal.pbio.1002378, 14:e1002378. PMID: 26871440
470	26.	Puglisi E, Pascazio S, Suciu N, Cattani I, Fait G, Spaccini R, et al. Soil enzymology: classical
471		and molecular approaches. Biology and Fertility of Soils. 2012; 48: 743-762.
472	27.	Song Y, Song C, Yang G, Miao Y, Wang J, Guo Y. Changes in labile organic carbon fractions
473		and soil enzyme activities after marshland reclamation and restoration in the sanjiang plain in
474		northeast china. Environmental Management. 2012; 50: 418-426.
475		https://doi.org/10.1007/s00267-012-9890-x.PMID: 22744158

476	28.	Gong J, Hou W, Liu J, Kamran M, Kong X, Wang L, et al. Effects of different land use types
477		and soil depths on soil mineral elements, soil enzyme activity, and fungal community in karst
478		area of southwest china. International Journal of Environmental Research and Public Health.
479		2022; 19. https://doi.org/10.3390/IJERPH19053120.PMID: 35270817
480	29.	Nannipieri P, Trasar-Cepeda C, Dick RP. Soil enzyme activity: a brief history and biochemistry
481		as a basis for appropriate interpretations and meta-analysis. Biology and Fertility of Soils. 2018;
482		54: 11-19. https://doi.org/10.1007/s00374-017-1245-6.
483	30.	Jin Y, Xiong Y, Sun X, Li J, Li X, He S, et al. Effects of combined application of chemical
484		fertilizer reduction with trichoderma organic fertilizer on yield and quality of pepper and
485		enzyme activities in rhizosphere soil. Journal of Sichuan Agricultural University. 2021; 02.
486		https://doi.org/10.16036/j.issn.1000-2650.2021.02.009. (in Chinese)
487	31.	Zhang X, Ma X, Zhao M, Zhang B, Chi J, Liu W, et al. H2 and H3 relaxin inhibit high glucose-
488		induced apoptosis in neonatal rat ventricular myocytes. Biochimie. 2015; 108: 59-67.
489		https://doi.org/10.1016/j.biochi.2014.11.004.PMID: 25446652
490	32.	Burke D, Weintraub M, Hewins C, Kalisz S. Relationship between soil enzyme activities,
491		nutrient cycling and soil fungal communities in a northern hardwood forest. Soil Biology and
492		Biochemistry. 2011; 43: 795-803. https://doi.org/10.1016/j.soilbio.2010.12.014.
493	33.	Zhou J, Jiang X, Zhou B, Zhao B, Ma M, Guan D, et al. Thirty four years of nitrogen fertilization
494		decreases fungal diversity and alters fungal community composition in black soil in northeast
495		China. Soil Biology and Biochemistry. 2016; 95: 135-143.
496		https://doi.org/10.1016/j.soilbio.2015.12.012.

497	34.	Hu X, Jiang Y, Shu Y, Hu X, Liu L, Luo F. Effects of mining wastewater discharges on heavy
498		metal pollution and soil enzyme activity of the paddy fields. Journal of Geochemical
499		Exploration. 2014; 147: 139-150. https://doi.org/10.1016/j.gexplo.2014.08.001.
500	35.	AOAC. Vitamin C (ascorbic acid) in vitamin preparations and juices. Official Methods of
501		Analysis. 1984; 1058-1059.
502	36.	Yang H, Huang Y. Fruit and vegetable products-determination of soluble solids content-
503		Refractometric method. Q.H.B 1990; 122: 90-95.
504	37.	AOAC. Official methods of analysis. Trac Trends in Analytical Chemistry. 1990; 15: 104-124.
505	38.	Miao J, Yang H, Li Q. Spectrophotometric determination of trace nitrites in food products.
506		Chinese Journal of Spectroscopy Laboratory. 2009; 26: 541-542. (in Chinese)
507	39.	Spiro RG. Analysis of sugars found in glycoproteins. In Methods in Enzymology, Academic
508		Press.1966; 8: 3-26.
509	40.	Tian C, Qiu T, Zhu F, Xiao J, Wei L, Liang Z, et al. Calcium oxide regulation of fusarium wilt
510		and rhizosphere bacterial community. Journal of Hunan Agricultural University. Natural
511		Sciences. 2018; 44: 620-624. https://doi.org/10.13331/j.cnki.jhau.2018.06.008. (in Chinese)
512	41.	Li J, Hou H, Wang C, Zhang S, Ma J, Ding Z, et al. Soil Bacteria Diversity of Reclaimed Soil
513		Based on High Throughput Sequencing. Environmental Science & Technology. 2018; 41: 148-
514		157. https://doi.org/10.19672/j.cnki.1003-6504.2018.12.022. (in Chinese)
515	42.	Chen D, Cheng J, Chu P, Hu S, Xie Y, Tuvshintogtokh I, et al. Regional-scale patterns of soil
516		microbes and nematodes across grasslands on the Mongolian plateau: relationships with
517		climate, soil, and plants. Ecography. 2015; 38: 622-631. https://doi.org/10.1111/ecog.01226.

518 Sun H, Yu L, Zhang Y. Recent advance in Geodermatophilaceae- A review. Acta 43. 519 Microbiologica Sinica. 2015; 55: 1521-1529. 520 https://doi.org/10.13343/j.cnki.wsxb.20150128.PMID: 27101693 521 Lee H, Lautenbach S, Nieto APG, Bondeau A, Cramer W, Geijzendorffer IR. The impact of 44. 522 conservation farming practices on Mediterranean agro-ecosystem services provisioning—a 523 meta-analysis. Regional Environmental 2019; 19: Change. 2187-2202. 524 https://doi.org/10.1007/s10113-018-1447-y. 525 45. Liu H, Han H, Wang Q, Zhuang H, Wang H. Effect of vegetables-tomato rotation on soil 526 microbial diversity, enzyme activity and physicochemical properties of vegetables in 527 Microbiologica 61: greenhouse. Acta Sinica. 2021; 167-182. 528 https://doi.org/10.13343/j.cnki.wsxb.20200139. (in Chinese) 529 46. Zhou J, Chen J H, Ma J Y, Xu N, Xin F X, Zhang W, et al. Luteimonas wenzhouensis sp. nov., 530 A chitinolytic bacterium isolated from a landfill soil. Current Microbiology. 2020; 78: 383-388. 531 https://doi.org/10.1007/s00284-020-02293-9.PMID: 33258058 532 47. Long L, Xu KW, Shen DY, Chou SH, Mark G, Oian G. Antifungal weapons of Lysobacter, a 533 mighty biocontrol agent. Environmental Microbiology. 2021; 23 (10): 5704-5715. 534 https://doi.org/10.1111/1462-2920.15674.PMID: 34288318 535 Wang S, Huang G, Li N, Liu L. The effects of paddy-upland rotation on microorganisms of soil 48. 536 (the Tird Year). Acta Agriculturae Universitatis Jiangxiensis. 2002; 24: 320-323. 537 https://doi.org/10.1007/s11769-002-0038-4. (in Chinese) 538 49. Qiu X, Peng D, Tian H, Wang H, Liu X, Cao L,et al. Soil ecoenzymatic stoichiometry and 539 microbial resource limitation driven by thinning practices and season types in Larix principis-

540 rupprechtii plantations in North China. Forest Ecology and Management. 2021; 482: 118880. 541 https://doi.org/10.1016/J.FORECO.2020.118880 542 50. Bending GD, Turner MK, Jones JE. Interactions between crop residue and soil organic matter 543 quality and the functional diversity of soil microbial communities. Soil Biology and 544 Biochemistry. 2002; 34: 1073-1082. https://doi.org/10.1016/S0038-0717(02)00040-8. 545 51. Wu F, Zhao F, Liu Y. On the reasons of continuous cropping obstacles in vegetable facility 546 gardening. Journal of Northeast Agricultural University. 2000; 31: 241-247. 547 https://doi.org/10.19720/j.cnki.issn.1005-9369.2000.03.007. (in Chinese) 548 52. Acosta-Martínez V, Pérez-Guzmán L, Johnson JMF. Simultaneous determination of β-549 glucosidase, β-glucosaminidase, acid phosphomonoesterase, and arylsulfatase activities in a soil 550 sample for a biogeochemical cycling index. Applied Soil Ecology. 2019; 142: 72-80. 551 https://doi.org/10.1016/j.apsoil.2019.05.001. 552 Zhang N. The Differences of effects on soil properties of facilities by two kinds of hydroponic 53. 553 and upland rotations. Dissertation, Yangzhou University: Yangzhou, China. 2014; (in Chinese) 554 54. Kang Y, Jing F, Sun WQ, Tan J, Ran H, Jiang GY. Effects of continuous cropping of processing 555 tomato on physical-chemical properties of and microbial biomass in the soil. Acta Pedologica 556 Sinica. 2016; 53: 533-542. (in Chinese) 557 Dai Y, Yan Z, Xie J, Wu H, Xu L, Hou X. et al. Soil bacteria diversity in rhizosphere under two 55. 558 types of vegetation restoration based on high throughput sequencing. Acta Pedologica Sinica. 559 2017; 54: 735-748. https://doi.org/10.11766/trxb201603150062. (in Chinese)