1   **Straw biochar increases the abundance of inorganic phosphate solubilizing**
2   **bacterial community for better rape (*Brassica napus*) growth and phosphate**
3   **uptake**

4   Bang-Xiao Zheng[1,2,3,4], Kai Ding[1*], Xiao-Ru Yang[1], Mohammed A.M. Wadaan[5], Wael
5   N. Hozzein[5], Josep Peñuelas[3,4], Yong-Guan Zhu[1,6]

6   *[1] Key Laboratory of Urban Environment and Health, Institute of Urban Environment,*
7   *Chinese Academy of Sciences, Xiamen 361021, PR China*;
8   *[2] University of Chinese Academy of Sciences, Beijing 100049, PR China*;
9   *[3] Consejo Superior de Investigaciones Científicas (CSIC), Global Ecology Unit,*
10   *Centre for Ecological Research and Forestry Applications (CREAF)–CSIC–*
11   *Universitat Autonoma de Barcelona (UAB), Bellaterra, 08193 Barcelona, Catalonia,*
12   *Spain*;
13   *[4] CREAF, Cerdanyola del Vallès, 08193 Barcelona, Catalonia, Spain*;
14   *[5] Bioproducts Research Chair, Zoology Department, College of Science, King Saud*
15   *University, Riyadh 11451, Kingdom of Saudi Arabia*;
16   *[6] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-*
17   *Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China.*

18   Emails:

19   Bang-Xiao Zheng: bxzheng@iue.ac.cn; b.zheng@creaf.uab.cat
20   Kai Ding: kding@iue.ac.cn
21   Xiao-Ru Yang: xryang@iue.ac.cn
22   Mohammed A.M. Wadaan: wadaan@ksu.edu.sa
23   Wael N. Hozzein: whozzein@ksu.edu.sa
24   Josep Peñuelas: Josep.Penuelas@uab.cat
25   Yong-Guan Zhu: ygzhu@iue.ac.cn

26

27   * Corresponding Author

28   Key Laboratory of Urban Environment and Health, Institute of Urban Environment,
29   Chinese Academy of Sciences, Xiamen 361021, People's Republic of China. Phone:
30   (+86) 592 6190560; Fax: (+86) 592 6190977.

Abstract

The direct application of inorganic-phosphate-solubilizing bacteria (iPSBs) for improving the efficiency of phosphorus (P) use leads to a low rate of bacterial survival. Biochar is a good inoculum carrier for microbial survival, and diverse feedstocks can have different effects. We generated an iPSB community using seven selected iPSB strains with various phylogenic taxonomies and P-solubilizing abilities. Biochar was then inoculated with the iPSB community and applied to soil in pots seeded with rape (*Brassica napus*). Growth of the rape for four weeks and the effects of biochars produced from six raw feedstocks, rice straw, rice husks, soybean straw, peanut shells, corn cobs and wood, were compared. The synthetic iPSB community had a larger capacity to solubilize inorganic P and exude organic anions than any of the individual strains. The structure of the iPSB community was analyzed by high-throughput sequencing four weeks after inoculation. All seven iPSB strains were detected, dominated by *Arthrobacter defluvii* 06-OD12. The abundance of the iPSB community was significantly correlated with rape biomass, P content and P uptake ($P < 0.05$). The biochar amendments conferred 6.86-24.24% survival of the iPSB community, with the straw biochars conferring the highest survival. The available-P content of the biochar rather than soil pH was the dominant factor for iPSB community structure, suggesting that the biochar material was critical for the survival and functioning of the iPSB community. Our study demonstrates the feasibility of biochar-assisted iPSB improvement of crop growth and P uptake.

Importance

Inorganic phosphate solubilizing bacteria (iPSBs) are commonly proved to be useful as biofertilizer for plant growth and phosphorus (P) uptake. Previous studies dedicated to screen iPSB isolates with high solubilizing capacity. However, the exact limitation would attribute to the low survival rate after direct inoculation, dramatically shortening functioning periods of iPSB strains. A synthetic iPSB community with 7 different generic strains were first-time applied with plant inoculation. By observing the survival condition, including abundance and community structure, the results proved the straw biochar, which compared with other 5 feedstock biochars, was optimal for iPSB community survival.

Introduction

Phosphorus (P) is an essential macronutrient for plant growth. P, however, is a non-renewable element, so the availability of rock P has been dramatically decreased to satisfy the increased demands for P fertilizers (Elser and Bennett, 2011; Elser et al., 2007; Penuelas et al., 2013). Applied P fertilizers are readily immobilized due to the strong fixation in soil solutions, which renders P unavailable for plant uptake and hinders the movement of P in terrestrial systems (Kochian, 2012). Developing new strategies to increase P-use efficiency are greatly needed.

Microorganisms are active participants in numerous biogeochemical cycles (van der Heijden et al., 2008). Inorganic-phosphate-solubilizing bacteria (iPSB) are major drivers of the biomobilization of soil P, which releases many organic ions or protons to effectively liberate immobilized P and preserve P for being fixed again (Richardson et al., 2009; Richardson and Simpson, 2011). iPSB microbes are also commonly accepted as plant-growth-promoting rhizobacteria (PGPR) and are commonly used as biological inoculants to improve P assimilation and plant productivity (Collavino et al., 2010; Kumar et al., 2012). The low survival rate of the bacteria after being introduced into the soil, however, prevents the sustainability of PGPR inoculation (Bashan et al., 1995; Malusá et al., 2012).

Biochar obtained by the pyrolysis of various raw feedstocks is commonly used as a stable and economic inoculum carrier (Sun et al., 2016). Generally, biochar showed beneficial effects on the abundance, diversity and functional improvement of inoculated microbial community (Lehmann et al., 2011). Biochar with internal

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87   porosity provides a protective habitat to prolong PGPR survival, and its strong ability

88   to absorb nutrients can sustainably support the growth of inoculants (Zimmerman et

89   al., 2011), which has been extensively demonstrated (Ahmad et al., 2015; Akhtar et

90   al., 2015). The biochar addition was proved to be of great benefits for regulating

91   phosphate-solubilizing bacteria community (Wei et al., 2018; Wei et al., 2016).

92   Biochars produced from different materials, however, differ in their ability to maintain

93   PGPR due to their different physicochemical properties (Cantrell et al., 2012). A

94   previous study demonstrated the strong influence of biochars with different

95   characteristics on PGPR survival after incorporation into soil (Khan et al., 2014). The

96   selection of biochars is therefore of great importance for PGPR inoculations.

97   We selected biochars pyrolyzed from six raw feedstocks for maintaining the

98   effect of iPSBs on P mobilization to improve plant growth: rice straw (RS), rice husks

99   (RH), soybean straw (SS), peanut shells (PN), corn cobs (CC) and wood (WD). The

100   growth of rape (*Brassica napus*) needs large amounts of P (Belimov et al., 2002) and

101   was thus chosen as the test plant. Rape growth and the P status of the plants and the

102   rhizospheric soil were determined. An iPSB community containing seven microbial

103   strains with varying characteristics was used as a microbial inoculant, and the

104   structures of the communities after application of the six inoculated biochars were

105   analyzed. The relationships between rape growth, biochar addition and iPSB

106   community structure are also discussed.

107

Materials and Methods

Soil sampling and characterization

Soil samples (0-15 cm layer) were collected in May 2015 after crop harvest from the Changshu AgroEcological Experimental Station in Jiangsu Province, China (31°32'N, 120°41'E). The soil contained 0.78% clay, 18.38% silt and 80.84% sand. The samples were air-dried, sieved (0.2 mm) and stored at 4 °C and -20 °C until use and analysis.

Soil pH was determined in a suspension (1:2.5 dry soil:sterilized water (w/v)) using a XL60 pH meter (Fisher Scientific, Asheville, USA). Soil moisture content (%) was calculated as the difference in weight between fresh soil and soil dried in an oven at 300 °C for 24 h. Carbon, nitrogen and sulfur contents were measured with an elemental analyzer (vario MAX CNS, Elementar, Hanau, Germany). P and available-P contents were determined after digestion by strong acid (sulfuric acid) and extraction by sodium bicarbonate using the molybdate-blue method (Murphy and Riley, 1962; Olsen et al., 1954; Parkinson and Allen, 1975), respectively. The acid-digested soils were further used to determine the concentrations of metals (potassium, calcium, sodium, magnesium, iron and aluminum) by inductively coupled plasma optical emission spectrometry (ICP-OES, Optima 7000DV, Perkin Elmer, Waltham, USA). The physical and chemical properties of the Changshu soil are listed in Table S1.

Experimental design and analysis

128   The diagram of experimental design was presented in Fig.1. In brief, the soil was

129   used for new iPSB strain isolation as well as rape growth with its sterilized version.

130   A common iPSB community is composed of strains from genus *Arthrobacter*,

131   *Streptomyces*, *Bacillus* and *Beta-* and *Gamma-proteobacteria* (Zheng et al., 2017).

132   Based on that, we compared the isolated iPSB strains with a previous isolated iPSB

133   database to select representative iPSB strains based on their morphology, taxonomic

134   diversity and biochemical characterization. Then, 7 representative iPSB strains,

135   including *Arthrobacter defluvii* 06-OD12 (KU647200), *Burkholderia cepacia* 51-

136   Y1415 (KU647244), *Bacillus megaterium* CS22 (MG430229), *Pseudomonas*

137   *frederiksbergensis* 11-D3 (KU647205), *Rhodanobacter* sp. 25-Y8 (KU647218),

138   *Streptomyces prasinopilosus* 34-Y1 (KU647227) and *Variovorax paradoxus* 19-D4

139   (KU647212), were chosen to generate iPSB community, which was validated by

140   biocompatibility and P solubilization capacity tests. Besides, six raw feedstocks,

141   including RS, RH, SS, CC, PN and WD, were used to form biochars. The pH, carbon

142   and nitrogen percentages, available P concentration, Brunauer-Emmett-Teller (BET)

143   surface area and pore size of biochar were determined. To see the effect of biochar on

144   iPSB community-assisted crop growth, six iPSB community-adhered biochars (3%)

145   were inoculated into Changshu soil with rape (*Brassica napus*) growth while a soil

146   with iPSB community inoculation but without biochar amendment was treated as

147   control (CK). Before and after 4-week rape growth, the pH, available-P concentration

148   and total P amount of rhizospheric soil, biomass, total P content and P uptake of plant

149   and iPSB community survival rate were measured. The iPSB community structure

150   was also analyzed by 16S rRNA high-throughput sequencing with iPSB database

151   alignment. Besides, to discriminately quantify the impact of biochar and iPSB

152   community on the plant growth and its P uptake, we conducted another 21-day rape

153   growth with four treatments: rape growth without biochar or iPSB (R-CK), only 3%

154   RS biochar addition (R-B), with only iPSB community inoculation (R-P) and with

155   iPSB community-adhered RS addition (R-BP). The P-related parameters were

156   measured before and after rape growth. The detailed explication could be found in

157   supplementary materials and methods.

158   *Statistical analysis*

159   One-way correlation and variance were determined using SPSS Statistics 21

160   (IBM, New York, USA). Heatmapping was generated by the PHEATMAP package

161   (http://rpackages.ianhowson.com/cran/pheatmap/) based on R studio (version 3.2.3).

162   Redundancy analysis (RDA), principle component analysis (PCA), variance partition

163   analysis (VPA) and Monte Carlo permutation test were also performed by using R

164   studio (version 3.2.3) with and VEGAN (Oksanen et al., 2007) packages.

Results

Physical and chemical properties of the biochars

All physical and chemical parameters of the six biochars were highly variable between treatments (Table S2). RS had the highest pH (10.30 ± 0.34), and RH had the lowest pH (8.92 ± 0.40) but the highest BET surface area (43.00 ± 3.47 m^2 g^{-1}). Pore size differed significantly among the biochars ($P < 0.05$). C content was significantly higher for CC (74.80 ± 0.87%) and WD (75.93 ± 5.86%) than the other biochars, and N and Olsen-P contents were highest for RS (2.22 ± 0.21% and 685.80 ± 52.14 mg kg^{-1}, respectively).

Selection and characterization of the iPSB strains

We selected seven iPSB strains based mainly on their P-solubilizing ability and phylogenic diversity to artificially simulate an iPSB community. We selected from 133 iPSB strains, 76 from a previous study and 57 from our study. *B. megaterium* CS22 (*Firmicutes*) had the highest hydroxyapatite content (139.35 μg mL^{-1}, Table S3) and ability to solubilize calcium-phosphate (Ca-P) (119.37 ± 2.80 μg mL^{-1}, Table S5) and was thus selected for the community. Six other strains from different classes with good Ca-P-solubilizing ability were also selected: *Rhodanobacter* sp. 25-Y8 (Gammaproteobacteria, 97.34 ± 2.00 μg mL^{-1}), *P. frederiksbergensis* 11-D3 (Gammaproteobacteria, 51.65 ± 16.41 μg mL^{-1}), *V. paradoxus* 19-D4 (Betaproteobacteria, 26.62 ± 4.00 μg mL^{-1}), *S. prasinopilosus* 34-Y1 (Actinobacteria,

185   17.93 ± 8.98 μg mL^{-1}), *B. cepacia* 51-Y1415 (Betaproteobacteria, 15.14 ± 1.03 μg

186   mL^{-1}) and *A. defluvii* 06-OD12 (Actinobacteria, 13.15 ± 0.17 μg mL^{-1}).

187   We comprehensively characterized these seven strains before generating the

188   iPSB community. They had various cellular sizes and shapes (0.5-10 μm and spherical

189   or rod-shaped, Fig. 2a). All these strains could survive in the LB, R2A and PVK (with

190   NH_4^+ or NO_3^- as the sole nitrogen source) media but with different enzymatic

191   activities and abilities to hydrolyze carbohydrates and form acids (Table S4). For

192   example, *B. cepacia* 51-Y1415 hydrolyzed 10 of 11 carbohydrates, and *B.*

193   *megaterium* CS22 hydrolyzed only seven but hydrolyzed potassium gluconate, which

194   is different from the other strains. *B. megaterium* CS22 could also form organic acids

195   from 23 of 49 organic compounds, but *Rhodanobacter* sp. 25-Y8 did not form

196   detectable amounts of any acid. The seven strains formed colonies with different

197   morphologies in LB medium (Fig. 2b). The experiments with bromocresol-purple

198   staining indicated that anion exudation differed substantially between strains that were

199   cultivated in PVK-NH_4^+ and PVK-NO_3 media (Fig. 2c, d). The pHs of the PVK media

200   and ability to secrete organic anions after 72 h of cultivation also varied among the

201   strains (Fig. 2e and Table S6). *B. megaterium* CS22 produced the lowest medium pH

202   (4.73) without secreting lactic, acetic or malic anions but secreted the most succinic

203   anions (196.60 ± 9.98 ppm). *A. defluvii* 06-OD12 produced the highest medium pH

204   (8.04) and secreted abundant organic anions and the most malic anions (107.33 ± 5.71

205   ppm).

Assessment of iPSB-community generation

207   The biocompatibility experiment by colony steaking indicated that these seven

208   strains survived well together (data not shown). The abilities of the community

209   generated by mixing the seven strains at the same cell density (10^9 CFU mL^{-1}) to

210   solubilize Ca-P (146.77 µg mL^{-1}) and ferric-phosphate (Fe-P) (29.16 µg mL^{-1}) were

211   significantly higher than those of the individual strains ($P < 0.05$, Table S5). The

212   features of community growth indicated an initial large release of inorganic P (78.28 ±

213   6.89 µg mL^{-1} at 12 h) and then a continuous increase to 146.77 ± 15.81 µg mL^{-1} after

214   144 h of cultivation. All six organic anions were detected from the cultivation of the

215   iPSB community (Table S6). Malic and lactic anions were the two most abundant

216   organic anions (80.48 ± 10.28 and 64.03 ± 5.94 µg mL^{-1} after 144 h, respectively),

217   which increased with the release of inorganic P.

218   *The bacterial, soil and plant responses after four weeks of rape growth*

219   Parameters of the microbes, rhizospheric soil and plants associated with the four

220   weeks of rape growth are presented in Table 1. The bacterial population was largest

221   for RS (2.88 ± 0.45 × 10^8 CFU g^{-1} dry soil), followed by RH and SS (1.89 ± 0.18 and

222   1.52 ± 0.39 × 10^8 CFU g^{-1} dry soil, respectively). iPSB survival after four weeks of

223   rape growth was significantly higher for RH (30.34 ± 1.49%) than CK, followed by

224   RS, SS and PN at 24.24 ± 2.15, 19.58 ± 1.29 and 18.79 ± 2.40%, respectively ($P <$

225   0.05). The pH of the rhizospheric soil was about 6.5-6.9 for all treatments. The total P

226   content was slightly higher for RH, CC and PN than CK. The amount of Olsen P,

227 however, was significantly higher with biochar addition and was highest for RS

228 (34.93 ± 0.72 mg kg^{-1}, $P < 0.05$). RS and SS had significantly higher plant biomass

229 (6.10 ± 0.57 and 6.14 ± 0.70 g pot^{-1}, respectively), P content (0.66 ± 0.03 and 0.68 ±

230 0.05 g kg^{-1}, respectively) and P uptake (4.05 ± 0.55 and 4.19 ± 0.36 mg pot^{-1},

231 respectively) than the other treatments ($P < 0.05$).

232 *Response of the iPSB community after four weeks of rape growth*

233   A total of 61816 reads of the 16S rRNA gene were obtained after filtering for

234 quality and removing chimeric reads. Of these, 15640 sequences with

235 similarities >99% with the iPSB database were accepted as potential iPSB species.

236 The percentage of iPSBs in each biochar treatment ranged from 6.44 ± 1.55% (CK) to

237 62.57 ± 17.27% (RS), with an average of 33.81 ± 18.58%. The abundance of the iPSB

238 community was significantly higher for RS and SS (50.73 ± 12.02%) than the other

239 biochar-addition treatments (Fig. 3a, $P < 0.05$), and the Shannon diversity index of the

240 community was significantly higher for CK (2.38 ± 0.01) and CC (2.36 ± 0.03) than

241 the other treatments (Fig. 3b, $P < 0.05$).

242   The pattern of the structures of the iPSB communities is shown in Fig. 4a. Thirty

243 species were aligned, and the seven selected iPSB strains were successfully detected

244 in all treatments. *A. defluvii* 06-OD12 was the dominant species with percentages of

245 the total iPSB populations of 1.04 ± 0.30, 4.61 ± 1.08, 3.99 ± 0.98, 3.13 ± 0.50, 38.28

246 ± 11.20, 9.65 ± 2.54 and 28.61 ± 7.77% for CK, WD, PN, CC, RS, RH and SS,

247 respectively. The average abundances of *B. megaterium* CS22, *B. cepacia* 51-Y1415,

248   *P. frederiksbergensis* 11-D3, *Rhodanobacter* sp. 25-Y8, *S. prasinopilosus* 34-Y1 and

249   *V. paradoxus* 19-D4 were 4.19 ± 2.23, 2.03 ± 1.83, 0.19 ± 0.25, 1.68 ± 0.65, $4.65 \pm$

250   3.14 and $2.09 \pm 2.14\%$, respectively. The PCA of different iPSB strains across

251   different biochar treatments showed that six strains from stimulated iPSB community

252   were well separated from other strains (Figure S1).

253   The RDA indicated that the first two axes explained 90.72% of the effect of the

254   soil and biochar characteristics on the iPSB community, with RDA1 and RDA2

255   explaining 85.07 and 5.65%, respectively (Fig. 4b). Biochar available P (AP) and soil

256   AP were the most important factors affecting the iPSB communities, supported by

257   their significant correlations ($P < 0.0001$) in the Monte Carlo permutation tests (Table

258   S7). Other significantly correlated factors were pore size, biochar N content, soil total

259   P content, biochar pH and BET surface area ($P < 0.0001$).

260   The contribution of each factor and their interactions to the structure of the iPSB

261   communities was quantified, and the percentages of the variance due to one or two

262   variables resolved by the VPA are listed in Table S8. Biochar and soil AP contributed

263   the most, at 0.352 and 0.219%, respectively. The interaction between soil AP and

264   biochar AP explained most of the variance (9.367%) for the iPSB community,

265   followed by the interactions between pore size and pH (3.273%) and between biochar

266   C and N content (3.045%). The analyses of correlations between iPSB community

267   abundance with diversity and the plant parameters are presented in Fig. 5. The

268   abundance of the iPSB community was significantly correlated negatively with its

269   diversity ($P < 0.001$) but positively with plant biomass, plant P and plant P uptake (P

270   < 0.001).

271   To discriminate the impact of biochar or iPSB community for the rape growth

272   and its P uptake, we conducted another experiment for 21 days. Results (Table S9)

273   showed that solo biochar addition (R-B) or iPSB inoculation (R-P) significantly

274   increased rhizosphere soil and plant P content and increased plant biomass compared

275   with R-CK treatment, while R-P was measured with significantly higher P amounts

276   than R-B in rhizosphere soil ($P < 0.05$). Combined amendment with biochar and iPSB

277   (R-BP) performed significantly higher soil P concentration and plant parameters ($P <$

278   0.05).

279

Discussion

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281   Plant biomass and the ability to take up P are thus important indices for assessing

282   the effect of an iPSB community on plant growth. Plant biomass, P content and P

283   uptake in our study were all significantly correlated ($P < 0.001$) with the abundance of

284   the iPSB community (Fig. 5b-d), suggesting that the inoculation of the biochar with

285   the iPSB community positively contributed to rape growth.

286   Inoculation of microorganism with biochar should perform with improvement

287   effect since the physical and chemical characters of biochar will provide appropriate

288   habitats for microbial community, including its abundance, diversity, nutrient

289   acquirement, mobilization and cycling (Lehmann et al., 2011; Quilliam et al., 2013).

290   All biochars tested in this study were beneficial as inoculum carriers for the survival

291   of the iPSB community, but their efficacy varied, perhaps due to the physical and

292   chemical properties of the individual biochars. RS was the best feedstock as an

293   inoculum carrier; the maintenance of the bacterial population was significantly

294   highest for RS ($2.88 \pm 0.45 \times 10^8$ CFU g^{-1} dry soil, $P < 0.05$; Table 1), perhaps due to

295   its surface area and porosity. BET surface area (35.29 ± 3.02 m^2 g^{-1}) and pore size

296   (27.86 ± 1.22 μm) were significantly higher ($P < 0.05$) for RS than the other biochars

297   except RH (Table S2). The larger pore size would provide a habitat niche for microbes

298   and have positive effects on the capacity to retain nutrients by binding both cations

299   and anions to the surface (Atkinson et al., 2010; Liang et al., 2006). A systematic

300   study of 32 biochars found that the survival of *Rhizobium tropici* had a quadratic

301   dependence on biochar pore size (Vanek et al., 2016).

302   The porosity of RS also allowed the retention of significantly ($P < 0.05$) more N

303   ($2.22 \pm 0.21\%$) and AP (685.80 ± 52.14 mg kg^{-1}) than the other biochars, which

304   provided a good habitat for the survival of the iPSB community. The correlation

305   analysis (Table S7) found that the structure of the iPSB community depended mostly

306   on biochar AP content ($R^2 = 0.881$), followed by biochar N content ($R^2 = 0.652$), pore

307   size ($R^2 = 0.656$) and BET surface area ($R^2 = 0.352$), suggesting that the survival of

308   the community may dependent on the retention of nutrients by the biochar. Biochar is

309   a good material for the retention of key macronutrients such N and P due to its surface

310   area and availability of anionic and cationic charges (Lehmann, 2007). The nutrient

311   status of biochars, however, depends on the origin of the biochar feedstock and its

312   mode of production. RS was the best feedstock in our study for nutrient storage and

313   bacterial survival. A previous study found that RS biochar could hold more organic C

314   and organic matter for the survival of *Bradyrhizobium* and sulfur-reducing bacteria

315   (Khan et al., 2014). Another study also reported that the benefits of RS biochar to

316   microbes contributed to the significantly higher survival than for other biochars of

317   *Bacillus mucilaginosus*, which had higher carbohydrate contents after four weeks of

318   incubation (Sun et al., 2015).

319   We used seven strains to generate the iPSB community. All strains were detected

320   by high-throughput sequencing after four weeks of cultivation (Fig. 4a). *A. defluvii*

06-OD12 was the most abundant iPSB strain, followed by *S. prasinopilosus* 34-Y1

and *B. megaterium* CS22. This finding was consistent with previous studies reporting

that *Arthrobacter*, *Streptomyces* and *Bacillus* were commonly isolated iPSB genera in

rhizospheric soils that promoted plant growth (Aislabie et al., 2006; Gopalakrishnan et

al., 2011; Taha et al., 1969; Xuan et al., 2011). *Pseudomonas* has been extensively

used as an iPSB genus due to a specific gene for solubilizing inorganic phosphate

(Kwak et al., 2015; Miller et al., 2010). *Pseudomonas* in our study, however, was less

competitive, perhaps because the biochars provided alkaline environments (pH >8,

Table S2), and *Pseudomonas* prefers acidic environments (Garbeva et al., 2004).

The RS and SS biochars that held abundant AP were the best for population

growth of the iPSB community (Fig. 4a). The size of the community was significantly

correlated with biochar properties and soil AP ($P < 0.05$, Fig. 4b and Table S7). The

interaction between soil and biochar AP contributed the most to the structure of the

community (Table S8). Soil pH has a strong impact on the abundance of bacterial

communities (Shen et al., 2013), and iPSB strains are sensitive to soil pH, with

abundance increasing with pH (Zheng et al., 2017). The interaction between soil pH

and pore size or N content of biochar would also make greater contribution for iPSB

community structuring, which may suggest that the combined effect of soil pH and

biochar would provide a better environment for the survival of iPSB community, even

for its better functioning. Our results, however, indicated that AP had a larger effect,

suggesting that the biochar provided a protective environment for the survival and

growth of the iPSB community. The combined effect of biochar and soil AP was

343   proved to be given the best contribution for the formation and functionality of iPSB

344   community, suggesting that available P was the critical dependent factor for iPSB

345   community. Exogenously amended inoculants may not survive better due to strong

346   competition with native microbes for long periods, so the storage of abundant

347   nutrients by biochars would be critical for increasing community size (Lehmann et al.,

348   2011). Biochars with nearly neutral internal pHs would benefit microbial survival,

349   suggested by the significant correlation between iPSB community abundance and

350   biochar pH rather than soil pH ($P < 0.05$). The biodiversity of the iPSB community

351   indicated by the Shannon index was significantly lower ($P < 0.05$) for RS and SS than

352   the other treatments (Fig. 4b), and the biodiversity of all treatments was significantly

353   negatively correlated ($P < 0.05$) with iPSB community abundance (Fig. 5a), perhaps

354   because the strains of the inoculated iPSB community survived well and became

355   dominant with the assistance of the RS and SS biochars.

356   The combined usage of RS biochar and iPSB community inoculation was most

357   beneficial for rape growth. In terms of rhizospheric soil (Table S9), RS biochar

358   significantly increased the P contents while the effect of iPSB community was much

359   higher ($P < 0.05$), suggesting that the iPSB community inoculation had the effect of P

360   mobilization and was better functioning on soil P releasing than biochar. As for the

361   impact on plant growth, we could not discriminate significant differences from the

362   effect of solo biochar or iPSB community on the promotion of plant biomass and its P

363   content and uptake; however, the combined application of biochar and iPSB

364   performed significantly higher promotion ($P < 0.05$), which suggested that the RS

365 biochar may provide a better habitat for P releasing by iPSB community to promote

366 rape growth. The biochar was extensively reported with beneficial effects on soil

367 nutrient mobilization and plant nutrient uptake (Atkinson et al., 2010), which was also

368 found in our study (nearly 2 times higher in soil Olsen-P concentration and 2 times of

369 plant P uptake). If we considered the enhancing effect of biochar for P releasing is

370 permanent, the remaining increasing effect observed in R-BP treatment (almost 1.5

371 times higher in available P enhancement and 1.5 times of plant P uptake than R-B

372 treatment) should be attributed to the functioning of iPSB community; however, the

373 actual effect of solo iPSB community inoculation was far less. One reasonable

374 explanation to this phenomenon may be that RS biochar provide a well environment

375 for iPSB survival and strengthen its ability for P mobilization. Although we have

376 found RS biochar was the best for the survival of iPSB community (Table 1), we

377 could not conclude that the biochar was the only reason for iPSB survival since there

378 is not a longer period (more than 3 month) to observe its living status. A similar study

379 of 20 weeks was reported that the biochar addition showed significant impact on the

380 population increase of *Bacillus mucilaginosus* within 4 weeks; however, the survival

381 rate was gradually declined afterwards (Sun et al., 2015). Another study also reported

382 the short-term effect of biochar amendment for the better survival of soil bacteria

383 (Hale et al., 2013). Both of those studies indicate that the biochar was effective but

384 may hardly give a long-term support for bacterial survival. A possibility is that there

385 would be not enough nutrients (such as available N and P) retained in biochar after a

386   long time, and the starving bacteria may enter into a non-culturable state to keep them

387   survive without functioning or even cell division (Sun et al., 2016).

388   The iPSB community benefited crop growth, and the biochar provided suitable

389   conditions for iPSB community survival due to its strong absorption of AP. All

390   biochar feedstocks in this study benefited the iPSB community, but the straw biochars

391   (RS and SS) were best for increasing iPSB community abundance and its functioning

392   in P mobilization.

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Figure legends

Figure 1. The diagram of experimental design. The soils sampled from Changshu was

used in new iPSB strain isolation and its sterilized one were used for rape growth with

different treatments. The asterisk (*) indicates that those samples were physically or

chemically analyzed. The pound sign (#) suggests that those strains were being

biochemically determined. The detail method was explicated in Supplementary

Information.

Figure 2. (a) Morphology of the iPSBs by scanning electron microscopy and colonies

after 72 h of cultivation at 30 °C on (b) LB medium, (c) PKV medium with NH_4^+ as the

N source and (d) PKV medium with NO_3^- as the N source. *E. coli* without P-solubilizing

ability was used as a negative control. (e) Heatmap of the profiles of medium pH and

organic-anion exudation by the iPSBs after 144 h of cultivation based on the average

of three replicate experiments.

Figure 3. Abundance (a) and Shannon diversity (b) of the biochars inoculated with the

iPSB community. Error bars represent the standard deviation of the replicates (n=4).

Different letters indicate significant differences at $P < 0.05$.

Figure 4. Heatmap and redundancy analysis (RDA) of the iPSB communities with the

biochars after four weeks of rape growth based on Bray-Curtis distances. (a) Profile of

iPSB community composition and (b) the effect of the physical and chemical properties

of the soil and biochar on the iPSB communities. The plotted values are natural-

544   logarithm transformations of relative iPSB community abundance. The columns in (a)

545   are labeled with biochar names and replicate numbers. The seven strains used to

546   generate the iPSB community are identified in red, and the other strains are identified

547   as species or genera.

548   **Figure 5.** Relationships between iPSB community abundance and the Shannon

549   diversity index (a), plant biomass (b), plant P (c) and plant P uptake (d).

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