

Effect of volatile organic compounds absorbed to fresh biochar on survival of *Bacillus mucilaginosus* and structure of soil microbial communities

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Abstract

Purpose Biochar is considered difficult for microorganisms to decompose, and volatile organic compounds (VOCs) sorbed to fresh biochar may affect the survival rate of inoculants or the structure of soil microbial communities. We tested the hypotheses that VOCs on fresh biochar may play a vital role in shaping the structure of soil microbial communities and determined if they inhibited or supported the growth of inoculants.

Materials and methods We examined the growth of *Bacillus mucilaginosus* in mushroom medium-based biochar (MM-biochar), corn stalk-based biochar (CS-biochar), and rice straw-based biochar (RS-biochar) in comparison with peat. The composition of VOCs before and after the incubation was characterized by pyrolysis-gas chromatography/mass spectroscopy (GC-MS). The structure of a soil microbial community incubated in biochar was examined via denaturing gradient gel electrophoresis (DGGE). Canonical correspondence analysis (CCA) was applied to reveal the contribution of pH, K and Na, and diversity indices from VOC fingerprints to diversity indices in DGGE profiles.

Results and discussion In the present study, all biochars were able to support *B. mucilaginosus* at population densities analogous to peat. Phenols comprise a fraction of the VOCs that potentially could be toxic to some microbes and inhibit their growth in the short time. The structure of the inoculated soil

microbial communities in terms of the diversity indices calculated from 16S ribosomal DNA (16S rDNA) and 18S rDNA DGGE profiles was greatly affected by biochar. Besides, CCA revealed the role of VOCs in shaping the structure of soil microbial communities.

Conclusions VOCs absorbed to biochar, despite their short life spans, could support the survival of *B. mucilaginosus*, demonstrating the potential of biochars as carriers for inoculants. The changes in the soil microbial communities induced by fresh biochar may not represent the long-term “biochar effect.” Therefore, future work needs to appreciate mechanisms underlying aged biochar on the structure of soil microbial communities.

Keywords Biochar · Inoculant · Soil microbial community · Volatile organic compound

1 Introduction

Biochar refers to organic matter, such as forestry and agricultural residues, pyrolyzed under oxygen deficit conditions (Lehmann 2007). The existence of Amazonian Dark Earths (ADEs) was recognized hundreds of years ago, yet only in the last decade has it received broad interest (Woods and Denevan 2009). While plant residues, mammal and fish bones, ash, and human excrement are also considered to be associated with the formation of ADEs, biochar is regarded as one of the most important components (Glaser and Birk 2012).

It has been reported that ADEs contain higher biomass and microbial diversity in comparison with the adjacent soils (Kim et al. 2007; Ruivo et al. 2009; O’Neill et al. 2009; Taketani et al. 2013). Among variables, e.g., current land use, soil texture, soil mineralogy, soil nutrient contents, or pH, biochar is proposed as the major driver affecting the bacterial

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community composition (Grossman et al. 2010). Soil microorganisms can oxidize biochar thus forming functional groups which can increase nutrient retention via ion exchange (Glaser et al. 2002) and, in general, stabilize soil organic matter (SOM) in ADEs (Glaser 2007). However, given to the complexity of geochemical and biological processes at the large time scale, it is still challenging to explain the “biochar effect” on the microbial population in ADEs.

A considerable number of studies have been conducted to examine the effect of fresh biochar on soil microbial communities (Jin 2010; Anderson et al. 2011; Chen et al. 2013a; Watzinger et al. 2013; Ng et al. 2013; Rutigliano et al. 2014). The structure of soil microbial communities in biochar and biochar-amended soils showed similarities with that of ADEs (Sun et al. 2013), but information is still insufficient to conclude the impact of various biochars on soil microbial communities, due to the profound dissimilarities between fresh and aged biochar (Neves et al. 2003).

Volatile organic compounds (VOCs) absorbed to fresh biochar are very likely influencing the soil microbiological and communities after application (Farrell et al. 2013). We tested the hypothesis that VOCs on fresh biochar may play a vital role in shaping the structure of soil microbial communities. Data suggest that some sorbed organic substances on biochar comprise a fraction of the VOCs that potentially could be toxic to some microbes and inhibit their growth (McClellan et al. 2007; Gurtler et al. 2014), and may shift the structure of soil microbial communities (Graber et al. 2010). Although VOCs on biochar have been qualified (Spokas et al. 2011; Hale et al. 2012), their functions on the structure of soil microbial communities are not well clarified.

An additional use of biochar may be as carrier for soil inoculants owing to its porosity and relative high nutrient content. Although peat and vermiculate have been shown suitable for growth of inoculants (Brockwell 1977), they are not abundant in some areas and unearthing them brings about detrimental environmental effects (Herrmann and Lesueur 2013). Biochar is prepared from abundant raw materials, sterilized during pyrolysis, and has the capability of adsorbing substrates needed by inoculants (Pietikäinen et al. 2000). Charcoal is made under similar process as biochar and acquires identical properties, having been suggested as well carriers for inoculants (Pietikäinen et al. 2000), but incorporation of it into soils is too expensive. Agricultural residuals have been promoted as feedstocks for biochar preparation, because of their widespread sources (Chen et al. 2013b). It has been reported that biochar could support growth of inoculants (Saranya et al. 2011), yet the role of VOCs on the survival of the inoculants is still unclear. We hypothesized that VOCs absorbed to the fresh biochar could either inhibit or support the growth of inoculants, depending on the composition and/or abundance of the VOCs.

To test our hypotheses, we inoculated *Bacillus mucilaginosus* onto three alkaline biochars with similar nutrient contents and examined the changes in VOCs on the fresh biochar during the inoculation period. In addition, we incubated a soil suspension with fresh biochar and assessed the structure of the soil microbial communities using denaturing gradient gel electrophoresis (DGGE). Pyrolysis-gas chromatography/mass spectroscopy has been suggested as a valid tool for detection and identification of VOCs derived from soil, food, compost, biowaste, etc. (Insam and Seewald 2010) and was applied to determine the fingerprints of VOCs in fresh biochar. Thereafter, the potential influence of physicochemical properties and VOCs absorbed to fresh biochar on the structure of soil microbial communities was determined by canonical correspondence analysis (CCA).

2 Materials and methods

2.1 Biochar characterization

The feedstocks used for biochar preparation were corn stalk, mushroom medium (residues left after mushroom harvest), and rice straw. Slow pyrolysis was carried out in a traditional biochar kiln (Jinhefu Ltd., China) at atmospheric pressure for 24 h. The feedstocks at the bottom of the kiln were burned to initiate the pyrolysis process and maintained at 450 °C for 12 h, respectively. We ground, sieved (2-mm mesh size), and mixed samples (biochar and peat) prior to the analysis of physical and chemical properties. Total C, N, S, and O were determined by Elementar Vario max Analyzer (Vario EL/micro cube, Elementar, Germany). Available N, Olsen P, and extractable K were analyzed according to soil agricultural chemistry analysis (Bao 1999). Ash content was determined according to GB/T 12496.4-1999, <http://down.foodmate.net/standard/sort/3/5367.html>. Volatile matter was determined according to GB/T 2001-91, <http://www.51zbz.net/biaozhun/61958.html>. The micronutrients were analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) (AA-7000, Shimadzu, Japan). The pH was determined in a soil and water suspension (ratio of 1:10) using by a compound glass electrode (PHS-4CT, Kang Yi Instrument Co., Ltd., China). Surface areas were determined by the Brunauer, Emmett, and Teller (BET) (V-Sorb 4800P, Gold Spectrum Technology Co., Ltd., China) method, and prior to BET analysis, the samples were degassed for 2 h at 105 °C. The water-soluble carbon (WSC) was determined using a TOC analyzer (3100, Shimadzu, Japan). Prior to the analysis, samples were stirred with distilled water (ratio of 1:20) for 24 h at room temperature, centrifuged at 10,000 rpm for 10 min, and filtrated through a 0.45-mm glass fiber. Also, from this extract, the water-soluble carbohydrates were measured by the

anthrone method and water-soluble phenols by the Folin method (Kuwatsova and Shindo 1973).

2.2 Analysis of volatile organic compounds

The composition of VOCs on the biochar was assessed using pyrolysis-gas chromatography/mass spectroscopy (GC-MS) (7890A, Agilent Inc., USA). Under high pyrolysis temperatures, secondary rearrangements may occur; therefore, it is difficult to distinguish whether the VOCs are breakdown products or originate from the raw material (Kaal and Rumpel 2009). Thus, we set the pyrolytic temperature at 100 °C to reduce secondary rearrangements. First, samples were pulse-pyrolyzed under temperatures starting from 40 to 100 °C at a rate of 20 °C s⁻¹ in quartz tubes on a Pyroprobe 5200 (CDS Agilent Inc., USA). When reaching the target temperature, it was kept for 15 s in order to detect products of pyrolysis processing. Then, pyrolyzed samples were transferred to a gas chromatograph at 280 °C for further separation by passing through a heated, fused silica capillary column (30 m × 0.25 mm). The GC oven temperature was increased from 40 °C for 2 min to 295 °C for 30 min at a rate of 8 °C min⁻¹. Mass spectra were obtained from 50 to 1,000 m z⁻¹ at a scan rate of 2,337.1 amu s⁻¹. Peaks were identified using the Automated Mass Spectral Deconvolution and Identification System (AMDIS V 2.65) and the National Institute of Standards and Technology (NIST) mass spectral library (Grandy et al. 2008).

2.3 Inoculation of carriers with *B. mucilaginosus*

Carriers were ground, sieved, and heated at 60 °C for 10 h. Peat was transferred into 250-ml Erlenmeyer flasks and autoclaved at 121 °C for 30 min. Finally, 100 g of the carrier medium was blended with 3 g of powder of *B. mucilaginosus* (a spore-forming plant growth promoting bacterium, purchased from Baoding Valley Biotechnology Co., Ltd., China) and incubated in 250-ml Erlenmeyer flasks under 28 °C. The water content was kept at 40 % of water holding capacity (WHC) by regular weighting and adding sterile water according to evaporation loss.

The population of *B. mucilaginosus* was enumerated by the serial dilution plate method. Briefly, 10 g of sample suspensions was treated at 80 °C for 10 min prior to plating to reduce interference of non-spore-forming bacteria. The suspension was diluted from 10⁻⁸ to 10⁻¹¹ and plated on petri plates containing broth medium with four replicates for each treatment. The cultures were incubated under 28 °C, and thereafter, the number of the inoculant colony-forming units (CFUs) was counted.

2.4 Setup of microcosms

Soils were sampled from a paddy field in Shenyang Agricultural University, Rice Research Institute, with sterile trowels and placed into sterile plastic bags, before being transported to the laboratory. The soil was diluted using sterile water at ratio 1:10 (w/w), and a small amount of sterile glass beads was added into the mixture. After vigorously shaking and filtering, equal amounts of the bacterial suspension from the soil were sprayed onto dried, sterile mushroom medium biochar (MM-biochar), corn stalk biochar (CS-biochar), rice straw biochar (RS-biochar), and control soil (sterile indigenous soil). The water content of all materials was adjusted to 40 % of WHC. Biochar carriers and the soil inoculated with the bacterial suspension were transferred into 250-ml Erlenmeyer flasks and incubated under 28 °C for 30 days.

2.5 DGGE procedures

Structures of bacterial and fungal communities in the soil suspension-inoculated biochar and indigenous soil were examined by DGGE. Whole-community DNA was extracted from 0.5-g samples using a Fast DNA[®] Spin Kit (Omega BioTek, USA) according to the manufacturer's protocol. The extracted DNA solutions were stored at -20 °C prior to polymerase chain reaction (PCR). Primers used in PCR amplification of 16S ribosomal DNA (16S rDNA) gene sequences are reported in Table 1. The PCR-DGGE profiles were generated using a D-Code system (Bio-Rad Laboratories, Inc.). The 8 % DGGE polyacrylamide gels (37.5:1, acrylamide/bisacrylamide) were prepared using a 45 to 70 % gradient (100 % denaturant contains 7 M urea and 40 % formamide) for separation of 16S rDNA fragments. As for separation of 18S rDNA fragments, the 10 % DGGE

Table 1 Primers used in this study for characterization of bacterial and fungal domains based on 16S rDNA and 18S rDNA, respectively

Primer name	Sequence (5'–3')	Reference
16S rDNA		
338F ^a	ACGGGGGGACTCCTACGGGAG GCA	Muyzer et al. (1993)
534R	ATTACCGCGGCTGCTGG	
18S rDNA		
ITS-2	GCTGCGTTCTCATCGATGC-3	Gardes and Bruns (1993)
ITS-1-F	CTTGGTCATTAGAGGAAGTAA	
ITS4 ^a	TCCTCCGCTTATTGATATGC	White et al. (1990)
ITS-F	ACGGGGGGCTTGGTCATTTAG AGGAAGTAA	

^a GC-clamp (CGCCCGCCGCGCGCGGGCGGGGCGGGGGG ACGGGGGG) was attached to the 5'-end of the primer

polyacrylamide gels were prepared with gradients ranging from 15 to 40 %. The DGGE was performed in TAE buffer at 60 °C and constant voltage of 60 V for 13 h. DGGE profiles were analyzed using Quantity One software (ver. 4.6.2). After background subtraction, frames and lanes were constructed on the DGGE profile, and Gauss Trace Qty was used to calculate band number, band position, and band intensity for each lane. The diversity of microbial communities was assessed in terms of richness S (band number) and Shannon-Wiener index as expressed by the equation $H = -\sum p_i \ln p_i$, where $p_i = n_i/N$, with n_i being the intensity of each band of a lane and N the sum of all band intensities in each lane. Cluster analysis was applied to represent the similarity of lanes in DGGE profiles, considering the report of Gauss Trace Qty.

2.6 Statistical analysis

A one-way ANOVA was used to compare the water-soluble carbon, carbohydrates, and water-soluble phenols and indexes representing the diversity of DGGE profiles and VOC fingerprints on biochar. Statistical analyses were conducted by Tukey's t test at the 5 % level with SPSS 11.5 (SPSS for Windows, Version 11.5, USA). The differences between values at $P > 0.05$ were considered not significantly different. A binary (1/0) matrix was conducted taking into account the presence or absence of individual substance in the fingerprints of VOCs on biochar. The matrix was further analyzed similar to the fingerprints in DGGE: richness S' (VOC number) and Shannon-Wiener index H' . CCA was carried out to assess the influence of selected physicochemical properties and VOCs absorbed to biochar on the structure of soil microbial communities.

3 Results

3.1 Physical and chemical properties

The mean particle diameter of MM-biochar (104.0 nm) was similar to that of peat (94.6 nm) and larger than that of CS-biochar (40.3 nm) and RS-biochar (54.2 nm). Moreover, the index of surface area and total porosity volume in RS-biochar was lower than that in CS-biochar, MM-biochar, and even in peat (Table 2).

Table 2 Particle size and specific surface area (BET) of materials

Parameter	Unit	CS-biochar	MM-biochar	RS-biochar	Peat
Mean diameter	nm	40.3 (0.3)	104.0 (3.5)	54.2 (0.6)	94.6 (0.9)
Surface area	m ² g ⁻¹	23.4 (0.2)	11.3 (0.3)	8.9 (0.2)	16.0 (0.2)
Total porosity volume	g cm ⁻³	0.25 (0.02)	0.24 (0.01)	0.12 (0.1)	0.32 (0.2)

Standard errors are in parentheses, $n=3$

The CS-biochar contained considerably more C (749.3 mg g⁻¹) than RS-biochar (566.1 mg g⁻¹), MM-biochar (410.0 mg g⁻¹), and peat (224.2 mg g⁻¹) (Table 3). In addition, MM-biochar had threefold more S than CS-biochar and RS-biochar. MM-biochar had more ash and volatile matter than CS-biochar and RS-biochar. As for macronutrients and micronutrients, most of them exhibited similar abundance in all biochars tested, but MM-biochar contained more S, Mg, and Ca, CS-biochar had more K, and RS-biochar had more Ni.

3.2 Water-soluble organic compounds

Prior to the inoculation of *B. mucilaginosus*, water-soluble carbon concentrations differed markedly between all materials, in order of RS-biochar (2,100 µg g⁻¹) < peat (3,800 µg g⁻¹) < MM-biochar (5,200 µg g⁻¹) < CC-biochar (7,700 µg g⁻¹). After the 20-week incubation, the content of water-soluble carbon declined significantly in all materials ($P \leq 0.05$, Fig. 1). Before the inoculation, the amount of carbohydrates was also similar in CS-biochar, MM-biochar, and RS-biochar (Fig. 2). The incubation of *B. mucilaginosus* resulted in decreases in carbohydrates in MM-biochar, and in CS-biochar and peat, the value was relatively stable, whereas in RS-biochar, the value increased fourfold (Fig. 2). Prior to the incubation test, the amount of water-soluble phenol was lower in peat than in biochar. At the end of the incubation experiment, the amount of water-soluble phenol had decreased in biochar treatments but increased statistically significant in peat ($P \leq 0.05$, Fig. 3).

3.3 Compositions of VOCs absorbed to biochar

At a pyrolysis temperature of 100 °C, more VOCs were detected in CS-biochar as compared to RS-biochar and MM-biochar (Fig. 4). The identified compounds corresponding to the pyrograms are shown in Fig. 5. It was also notable that most phenols were detected in CC-biochar, while 3-methylphenol and phenol were observed in MM-biochar and RS-biochar, respectively.

Overall, 41 peaks occurred in CS-biochar, 20 in MM-biochar, and 25 in RS-biochar (Fig. 6). Peaks detected in chromatographic profiles were submitted to AMDIS and NIST search. Among these substances, eight compounds were shared by CS-biochar, MM-biochar, and RS-biochar:

Table 3 Chemical properties of CS-biochar, MM-biochar, and RS-biochar and peat

Parameter	Unit	CS-biochar	MM-biochar	RS-biochar	Peat
TC	mg g ⁻¹	749.3	410.0	566.1	224.2
TN	mg g ⁻¹	13.4	14.4	13.6	13.6
C/N	–	55.9	28.5	41.6	16.5
TS	mg g ⁻¹	5.9	16.5	5.7	1.9
TO	mg g ⁻¹	128.1	202.5	112.9	–
Ash	mg g ⁻¹	155.7	336.6	210.6	–
VM	mg g ⁻¹	219.4	332.1	164.5	–
Avai.N	mg kg ⁻¹	0.75	0.38	0.22	0.55
Ols.P	mg kg ⁻¹	2.11	0.19	0.98	0.062
Extrac.K	mg g ⁻¹	13.4	4.01	8.12	0.77
pH _(1:10 Water)	–	9.2	7.9	7.9	–
Na	mg g ⁻¹	0.25	0.45	2.87	–
Mg	mg g ⁻¹	12.53	42.10	10.36	–
Al	mg g ⁻¹	2.01	4.99	2.31	–
Si	mg g ⁻¹	2.07	1.38	0.58	–
K	mg g ⁻¹	13.41	4.01	8.12	–
Ca	mg g ⁻¹	5.80	5.87	4.98	–
Fe	mg g ⁻¹	1.14	3.46	1.84	–
Ba	mg g ⁻¹	0.06	0.35	0.096	–
Pb	mg g ⁻¹	0.0002	0.009	0.003	–
Cr	mg g ⁻¹	0.012	0.011	0.11	–
Mn	mg g ⁻¹	0.15	0.55	1.54	–
Ni	mg g ⁻¹	0.007	0.008	0.097	–
Cu	mg g ⁻¹	0.023	0.031	0.015	–
Zn	mg g ⁻¹	0.05	0.09	0.12	–
B	mg g ⁻¹	0.02	0.05	0.04	–

TC total carbon, TN total nitrogen, TS total sulfur, TO total oxygen, VM volatile matter, Avai.N available nitrogen, Ols.P available phosphorus, Extrac.K extractable potassium

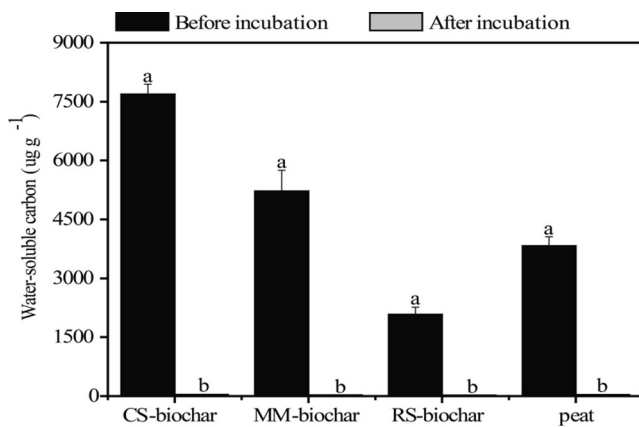


Fig. 1 Water-soluble carbon (WSC) in biochar and peat before and after the incubation of *Bacillus mucilaginosus*. Statistically significant differences by Tukey’s *t* test ($P \leq 0.05$) are denoted by different lowercase letters. Values are mean ($n=4$) \pm S.E. (bars). MM-biochar, CS-biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

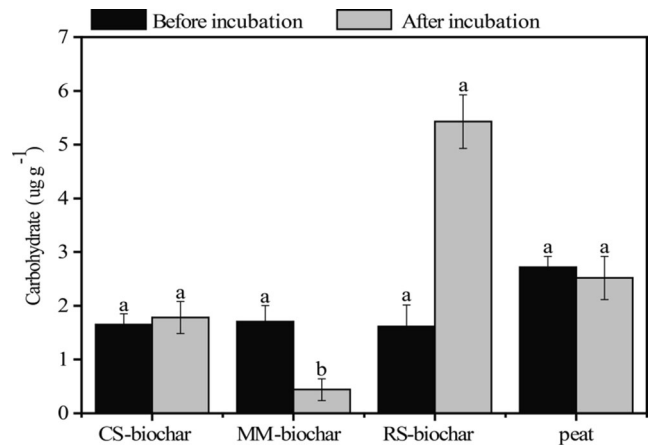


Fig. 2 Carbohydrate in biochar and peat before and after the incubation of *Bacillus mucilaginosus*. Statistically significant differences by Tukey’s *t* test ($P \leq 0.05$) are denoted by different lowercase letters. Values are mean ($n=3$) \pm S.E. (bars). MM-biochar, CS-biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

digitoxin, aldosterone, vitamin A aldehyde, 1-nonadecene, 1-tetradecanol, prednisolone acetate, dehydrocholic acid, and cortisone.

3.4 Survival of *B. mucilaginosus* on different biochars

Survival rate of the *B. mucilaginosus* in biochar and peat during the incubation period is shown in Fig. 7. Recovery of inoculated cells in all carriers occurred at week 2. After a 4-week incubation period, the number of CFUs detected from RS-biochar was higher than in other materials and lowest in the CS-biochar ($P \leq 0.05$). *B. mucilaginosus* survival declined in carriers from week 5 to 13, where it reached levels in the order of about \log_{10}^{10} cfu g⁻¹ in CS-biochar. At the final sampling time, the abundance of *B. mucilaginosus* in all

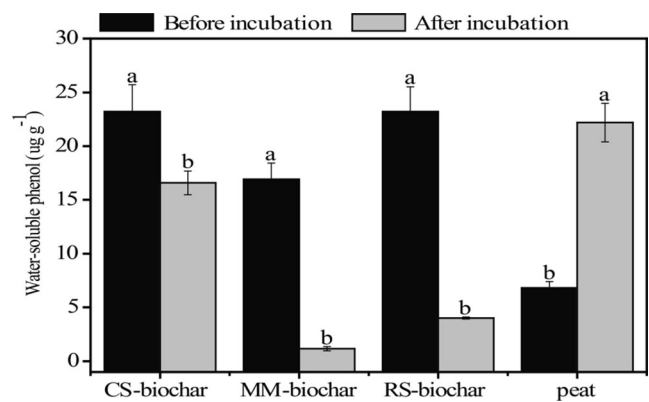


Fig. 3 Water-soluble phenol in biochar and peat before and after the incubation of *Bacillus mucilaginosus*. Statistically significant differences by Tukey’s *t* test ($P \leq 0.05$) are denoted by different lowercase letters. Values are mean ($n=3$) \pm S.E. (bars). MM-biochar, CS-biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

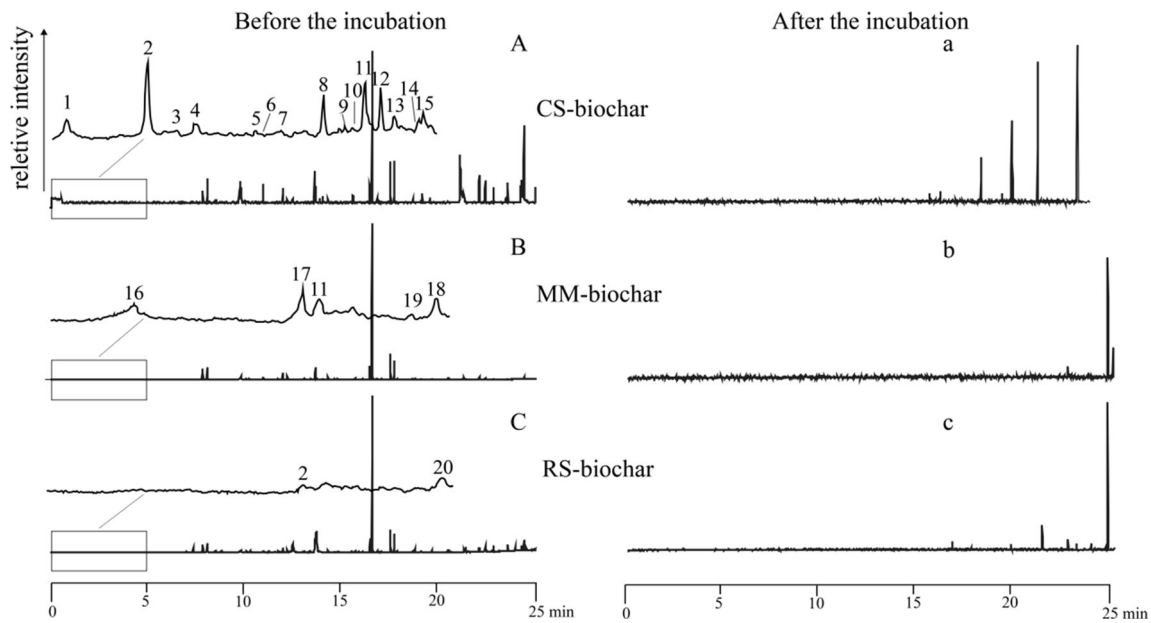


Fig. 4 Chromatograms of CS-biochar, MM-biochar, and RS-biochar analyzed at 100 °C with retention time from 0 to 25 min. Numbers correspond to pyrolysis products (retention time from 0 to 5 min) depicted in Fig. 5. A, B, and C indicate chromatographic profiles of materials prior

to the incubation of *Bacillus mucilaginosus*; a, b, and c show profiles after the incubation. MM-biochar, CS-biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

materials was lower than that in the first week: CS-biochar exhibited similar numbers of cells to MM-biochar and peat,

whereas that in RS-biochar was the lowest, less than $\log_{10}^{8.8} \text{ cfu g}^{-1}$ ($P \leq 0.05$).

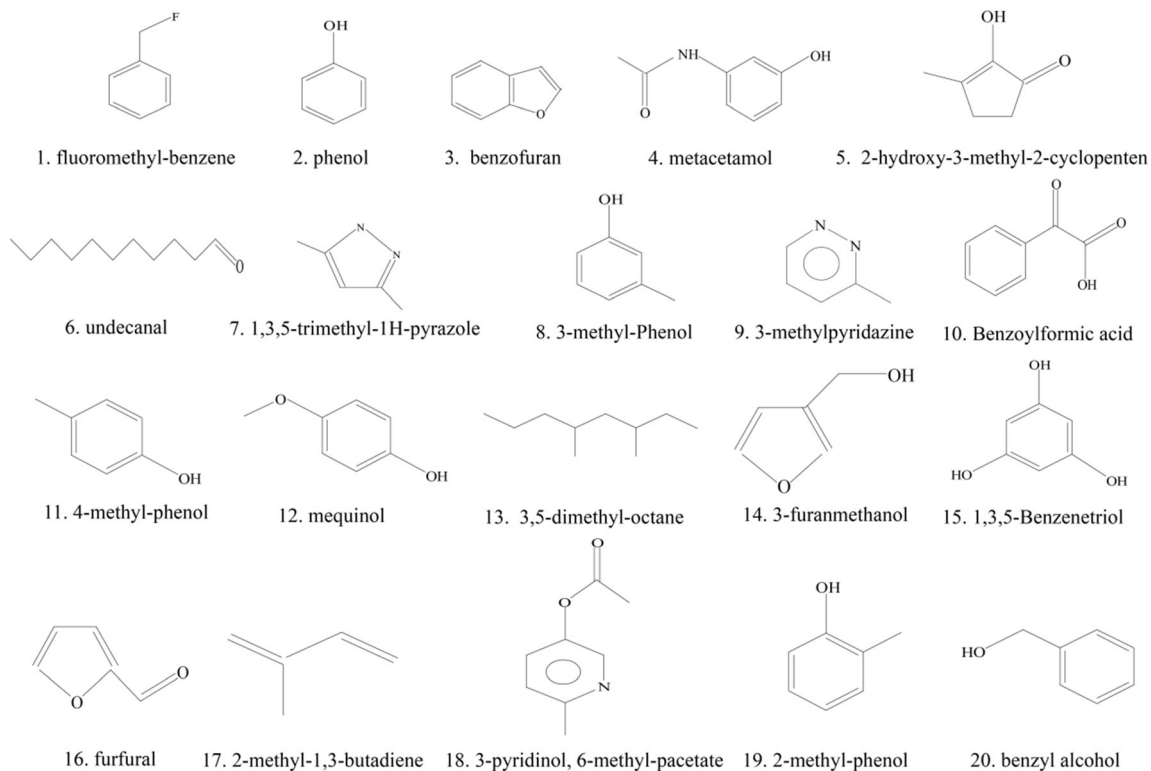


Fig. 5 Selection of pyrolysis products (retention time from 0 to 5 min) observed in CS-biochar, MM-biochar, and RS-biochar. Compound numbers refer to the peaks in the spectra of Fig. 4. MM-biochar, CS-

biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

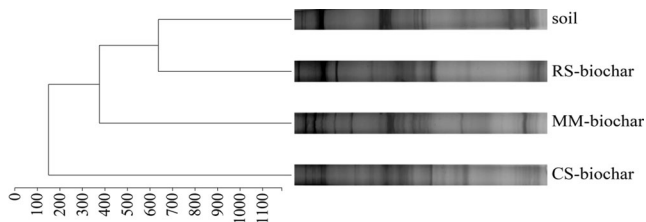


Fig. 9 Dendrograms of 18S rDNA DGGE profiles in the soil suspension-inoculated soil (indigenous soil), RS-biochar, MM-biochar, and CS-biochar. MM-biochar, CS-biochar, and RS-biochar represent mushroom medium-based biochar, corn stalk-based biochar, and rice straw-based biochar, respectively

profiles (both 16S and 18S), and Shannon-Weiner index (H') and richness (S') in the VOC fingerprints. The total variation can be explained by two axes: 79.8 % in axis 1 and 20.2 % in axis 2 (Fig. 10).

The index of S (16S) (1.14) was best correlated with axis 1, and H (16S) (1.44) and H (18S) (3.62) were profoundly correlated with axis 2. It is regarded that indices are significantly influenced when their values exceed 1. Hence, S (16S), H (16S), and H (18S) were significantly affected variables. The dominant driver on S (16S) was Na; meanwhile, pH, S' , H' , and K were dominant drivers on H (16S) and H (18S).

4 Discussion

In the present study, all biochars were able to support *B. mucilaginosa* at population densities analogous to peat, indicating the potential of biochar as carriers for inoculants. Prayogo et al. (2013) also observed that biochar addition increased abundance of Gram-negative bacteria and actinomycete by phospholipid fatty acid analysis. In addition, Watzinger et al. (2013) observed the incorporation of biochar into actinomycetal PLFA 10Me18:0 and Gram-negative bacterial PLFAs (16:1 ω 7c, 16:1 ω 5c, 18:1 ω 7c) and Me16:0 & i17:1 ω 8 and i17:0 in a 5-week trial.

Without soil incorporation, only the original properties of biochar should affect the growth of the inoculant, as opposed to environmental influences such as adsorption of organic compounds from soils. The mean particle diameter might affect O₂ availability, but their differences are inconsistent with the changes of *B. mucilaginosa* numbers in biochar, e.g., mean particle diameter in MM-biochar is about two times as much as that in CS-biochar and RS-biochar, but there was no corresponding changes in abundance of *B. mucilaginosa*. Available inorganic nutrients in biochar are also likely to affect the growth of the inoculant. However, CS-biochar only supported larger population densities of *B. mucilaginosa* at the final incubation time, despite the higher content of available N, Olsen P, and extractable K, which implies that available inorganic nutrients were not early limiting factors on the survival of the inoculant (Fig. 7).

The sharp decline in WSC in biochar and peat not only indicates the consumption of carbon by *B. mucilaginosa* but also explains the decrease in inoculant numbers. The result was in line with the report that biochar application stimulated soil microbial activity for only short periods (Rutigliano et al. 2014). Luo et al. (2012) recognized that pyrolysis temperatures influenced WSC in biochar, but our study also emphasizes the influence of the raw materials on this parameter. Even under the same pyrolysis condition, CS-biochar, RS-biochar, and MM-biochar exhibited differed carbon composition (ash and volatile matter), which may imply different pyrolysis degree among these biochars. It is reasonable that biochar contained fewer carbohydrates in comparison with peat because of the pyrolyzed process. However, the value of carbohydrates in RS-biochar increased during the incubation, which may imply the decomposition of organic matter insufficiently pyrolyzed in the traditional kiln. Likewise, it has been reported that a fraction of carbohydrates were detectable in a fast pyrolysis biochar (Bruun et al. 2012).

In the first 13 weeks of the incubation period, the CFUs of *B. mucilaginosa* obtained from CS-biochar were lower than that from RS-biochar, which seems contradictory to the WSC concentrations. This phenomenon might be explained by the

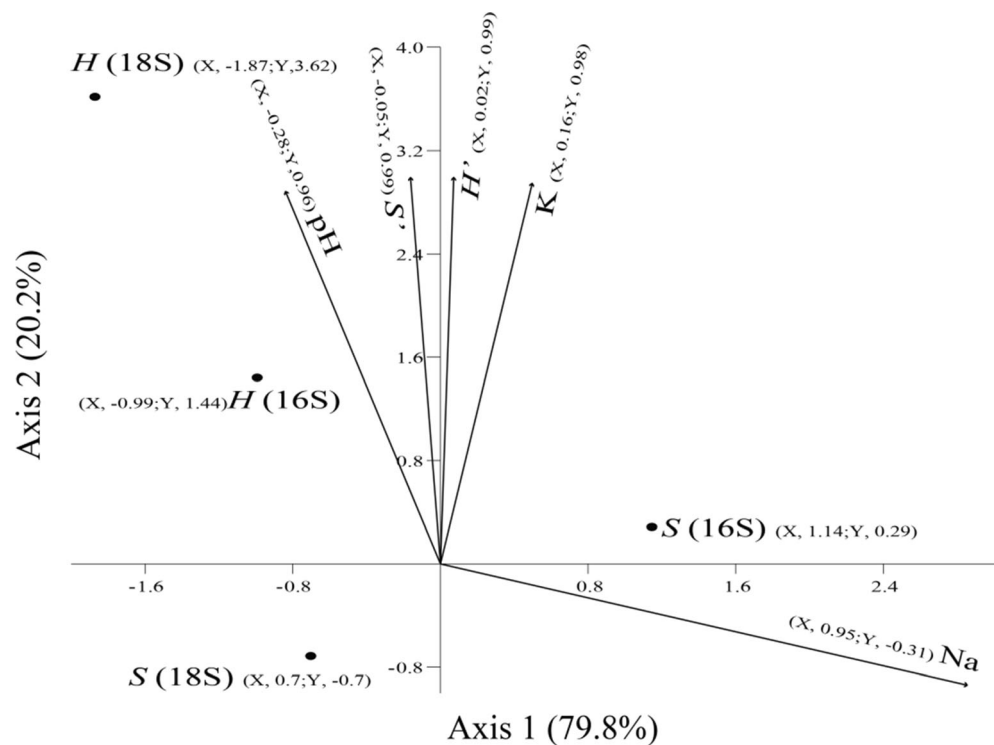
Table 4 Diversity indices estimated from DGGE profiles derived from the structure of soil microbial communities in the microcosms after 30 days of incubation and VOC fingerprints in biochar

Treatment	16S- S'	16S- H'	18S- S'	18S- H'	GC-MS- S'	GC-MS- H'
CS-biochar	38 ^a (2)	2.9 ^b (0.2)	31 ^b (1)	3.8 ^a (0.2)	41 ^a (3)	3.7 ^a (0.2)
MM-biochar	28 ^b (1)	3.1 ^a (0.1)	39 ^a (2)	3.6 ^b (0.3)	20 ^b (1)	3.0 ^c (0.1)
RS-biochar	26 ^b (2)	3.1 ^a (0.2)	39 ^a (3)	3.2 ^c (0.2)	25 ^b (2)	3.2 ^b (0.3)

Different superscript letters indicate significant differences between treatments (one-way ANOVA, LSD test, $P < 0.05$). Standard errors are in parentheses, $n = 3$

16S- S' richness of 16S rDNA profiles, 16S- H' Shannon-Wiener index in 16S rDNA profiles, 18S- S' richness of 18S rDNA profiles, 18S- H' Shannon-Wiener index in 18S rDNA profiles, GC-MS- S' richness of VOCs fingerprints, GC-MS- H' Shannon-Wiener index in VOCs fingerprints, MM-biochar mushroom medium-based biochar, CS-biochar corn stalk-based biochar, RS-biochar rice straw-based biochar

Fig. 10 Canonical correspondence analysis (CCA) reveals the influence of physicochemical parameters (pH, Na, and K) of biochar, variables (S' and H') from VOC fingerprints on indices (S and H) in DGGE profiles. S' = richness of VOC fingerprints; H' = Shannon-Wiener index in VOC fingerprints; S (16) = richness of 16S rDNA profiles; H (16) = Shannon-Wiener index in 16S rDNA profiles; S (18) = richness of 18S rDNA profiles; H (18) = Shannon-Wiener index in 18S rDNA profiles



phenols (e.g., phenol and 3-methyl-phenol) detected in CS-biochar, as their potential toxicity (Fritze et al. 1998; Villar et al. 1997) could inhibit the growth of the inoculant. Similarly, it was reported that some fast pyrolysis and slow pyrolysis biochar could inactivate enterohemorrhagic *Escherichia coli* O157:H7 in soil (Gurtler et al. 2014). This hypothesis was further supported by the observation that when VOCs including phenols in CS-biochar were consumed by the inoculant during the incubation, the abundance of *B. mucilaginosus* in CS-biochar was higher than that in RS-biochar. Phenols (e.g., 2-methyl-phenol and phenol) found in MM-biochar and RS-biochar might be in low abundance and did not exhibit obvious suppressive effects on the inoculant.

As biochar was selected as the sole medium for the incubation of soil suspension, the original properties of biochar itself should be considered as influencing the structure of soil microbial communities, irrespective of the complex interactions between soil and biochar. The structure of the inoculated soil microbial communities was greatly affected by biochar, in terms of changes in the diversity indices calculated from 16S rDNA and 18S rDNA DGGE profiles. As the most profound differences between biochar were pH, concentrations of Na and K, and fingerprints of VOCs, these factors were selected as potential variables influencing the structure of soil microbial communities and subjected to the CCA. CCA revealed the contributions of VOCs, pH, and Na as well as K on the microbial diversity indices, which supports our second hypothesis that VOCs play a vital role in shaping the structure of soil microbial communities. Besides, Ng et al.

(2013) found a tight relationship between composition of carbon and microbial community structure. Thereafter, Farrell et al. (2013) also noticed a significant change in microbial community induced by ^{13}C -labeled biochars, wherein Gram-positive bacteria utilized a considerable proportion of the biologically available fraction of biochar in a short period.

5 Conclusions

The overall data suggest that VOCs absorbed to biochar, despite their short life spans, could support the survival of *B. mucilaginosus*, demonstrating the potential of biochars as carriers for inoculants. Phenols comprise a fraction of the VOCs that potentially could be toxic to some microbes and inhibit their growth in the short time. CCA helped to find the role of VOCs absorbed to biochar in shaping the structure of soil microbial communities. The changes in the soil microbial communities induced by fresh biochar may not represent the long-term biochar effect as VOCs absorbed to biochar are likely metabolized by microorganisms over a very short period. Therefore, future work needs to appreciate mechanisms underlying aged biochar on the structure of soil microbial communities.

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