Applied Soil Ecology 110 (2017) 34–42

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases

Jinguang Liu[a,b], Xiaogang Li[a], Zhongjun Jia[c], Taolin Zhang[a], Xingxiang Wang[a,d,*]

[a] Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
[d] Jiangxi Key Laboratory of Ecological Research of Red Soil, Experimental Station of Red Soil, Chinese Academy of Sciences, Yingtan 335211, China

ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 19 October 2016
Accepted 3 November 2016
Available online 9 November 2016

Keywords:
Benzoic acid
Soilborne disease
Bacterial and fungal community
Fusarium
Burkholderia

ABSTRACT

As potent allelochemicals, phenolic acids are believed to be associated with soilborne diseases, and can influence plant-microbe interactions. Benzoic acid (BA) is one major phenolic acid found in peanut (*Arachis hypogaea*) root exudates. The objectives of this study were to estimate the BA degradation in the soil and its effects on soil bacterial and fungal communities and to detect the specific taxa responding to BA amendment. BA degradation was investigated by monitoring the BA retained in the soil using high-performance liquid chromatography (HPLC) and the CO_2 production rate using gas chromatography (GC). The abundance and diversity of the bacterial and fungal communities were investigated by quantitative real-time PCR (qPCR) and Illumina MiSeq sequencing. The results showed that the BA concentration decreased significantly with an increased rate of CO_2 production during the first 36 h after amendment, implying that the BA in the soil was quickly metabolized by the microbes. Quantitative PCR analysis further detected a significant increase in soil bacterial and fungal abundances in response to BA addition, but a reduced bacteria-to-fungi ratio. As a result of BA amendment, the relative abundance of *Fusarium*, *Bionectria* and *Trichoderma* was markedly increased, whereas *Metarhizium* was reduced. Moreover, BA (0.1 mmol L^{-1}) promoted the mycelial growth, sporulation capacity and conidial germination of the peanut root rot pathogen *Fusarium* sp. *in vitro*. Among bacteria, the relative abundance of *Betaproteobacteria* was significantly increased in response to BA treatment, whereas the relative abundances of *AD3* and *Actinobacteria* were reduced. A deeper taxonomic analysis of the *Betaproteobacteria* taxa showed a great increase in the abundance of the genus *Burkholderia*, from 0.71% to 10.12%, in response to BA amendment. Constructing clone libraries with the partial 16S rRNA genes of *Burkholderia* further demonstrated that BA amendment had modified the *Burkholderia* species composition. Our results highlight that the effects of BA in the soil are reflected by changes in populations of soil microbes and suggest that the response of specific microbes such as *Fusarium* and *Burkholderia* to BA might be associated with the development of soilborne diseases in monocultures.

1. Introduction

In negative plant-soil feedback (NF), conspecifics make soil conditions less suitable for themselves (Bever et al., 2012; van der Putten et al., 2013). The prevalence of soilborne diseases in monoculture is a typical example of NF and has caused great reductions in crop yield (Huang et al., 2013). Due to industrial demand and farmer cultivation habits in China, intensive monoculture has increasingly occurred with certain cash crops such as soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), watermelon (*Citrullus lanatus*), cucumbers (*Cucumis sativus*) and bananas (*Musa* spp.), which has resulted in severe occurrences of soilborne diseases (Li et al., 2014b; Ling et al., 2013; Wang et al., 2015; Zhou et al., 2014). To maintain yields, other practices, such as increasing pesticides and chemical fertilizers, have been applied, which has increased the input costs and constrained sustainable agricultural management; however, the mechanisms underlying the prevalence of soilborne diseases are not fully understood (Jordan et al., 2002; Wang et al., 2015; Zhou et al., 2014).

* Corresponding author at: Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China.
E-mail address: xxwang@issas.ac.cn (X. Wang).

http://dx.doi.org/10.1016/j.apsoil.2016.11.001
0929-1393/

As a major class of allelochemicals in the soil, phenolic acids are the primary polyphenols made by plants, and have multiple roles in plant-microbe interactions (Mandal et al., 2010; Singh et al., 1999). Studies have found that phenolic acids have toxic effects on the growth of plants and may be involved in the development of soilborne diseases (Baziramakenga et al., 1994; Inderjit Saini and Kaur, 2005; Bhowmik, 2004). In these studies, the effects of particular phenolic acids were primarily based on experiments without considering the soil microbial component, and high concentrations of active phenolic acids were often applied that may not be relevant to actual field situations (Bhowmik, 2004; Inderjit Saini and Kaur, 2005). When added to the soil, phenolic acids were rapidly metabolized by soil microorganisms and promoted the incidence of Fusarium wilt caused by *Fusarium oxysporum* in cucumber and watermelon (Blum, 1998; Wu et al., 2008; Ye et al., 2004; Zhou and Wu, 2012).

Peanut yield was generally decreased with increasing monocropping years, accompanied by the increasingly severe of soilborne diseases (Li et al., 2012; Li et al., 2014c; Wang and Chen, 2005). According to field investigation, yields of peanut were decreased by 28.9% and 51.2%, and root rot disease caused by *Fusarium* spp. increased from 8.1% to 17.4% and 54.9% in the 10 and 21 years monocropping fields, respectively, compared with the 3 years monocropping field. Various strategies, including crop rotation, application of chemical fungicides and organic manure amendments, have been proposed to alleviate the soilborne diseases (Jordan et al., 2002; Liu et al., 2015; Wang and Chen, 2005; Wang et al., 2015). Yet few research findings have demonstrated the successful increase of peanut yield and the control of peanut soilborne diseases under large scale field application conditions. During the root *Fusarium* infection, the concentration of phenolic acids in the root tissues and exudates was increased to inhibit the fungal procession (Lanoue et al., 2010; Michielse and Rep, 2009; Michielse et al., 2012). However, effects of phenolic acids on the soil suppressiveness towards peanut root rot disease still are not well understood.

Many soil microorganisms, including both bacteria and fungi, are reported to have pathways that degrade phenolic acids (Michielse et al., 2012; Pumphrey and Madsen, 2008). Using the plate counting method, the population of *F. oxysporum* in the soil was significantly increased in the presence of *p*-coumaric acid (Zhou and Wu, 2012). When *p*-coumaric acid or vanillic acid was artificially applied to soils, shifts in the microbial community of the rhizosphere were also detected using denaturing gradient gel electrophoresis (DGGE) or clone library methods (Zhou and Wu, 2012, 2013). Monoculture should dominate the development of soil microorganisms, due to a continuous release of phenolic acids into the soil. Associations between phenolic acids and peanut root rot disease need to be better understood in terms of soil microbial ecology (Li et al., 2014b; Zhao et al., 2015).

Intensive peanut monoculture in the hilly red soil regions of subtropical China has caused a significant decline in crop yield and quality and has increased susceptibility to root rot disease (Li et al., 2012; Liu et al., 2015; Wang and Chen, 2005). BA is one of the dominant phenolic acids in peanut root exudates, but its concentration is very low in peanut monoculture soil (Li et al., 2010, 2013, 2014a). We hypothesized that BA could be quickly metabolized by specific soil microbes and thus influence the composition of microbial community. The objectives of this study were as follows: (1), investigate the degradation of BA in the red soil; (2), evaluate its effects on the soil microbial community using high-throughput sequencing of the 16S rRNA and internal transcribed spacer 1 (ITS1) genes and to detect the specific taxa responding to BA treatment.

2. Materials and methods

2.1. Soil sampling

Soil was collected from a fallow agricultural field at the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, Yujiang County, Jiangxi Province, China (28°13′N and 116°55′E), where the monthly mean temperature varies from 5.9 °C in January to 30 °C in July. The annual precipitation is 1750 mm (averaged over 50 years). The selected field was previously planted with peanuts for three years and has then been kept fallow in the last five years.

Soil was collected from the surface layer (0–20 cm) and sieved (2 mm) to remove stones and plant residues. Some soil samples were air-dried to determine basic properties, and the others were stored at 4 °C until the experiments began. The soil was classified as Udic Ferrosol [FAO (1998) classification]. The soil had a pH_{H2O} of 4.78 and contained 7.63 g kg^{-1} of organic carbon, 0.83 g kg^{-1} of total nitrogen (N), 0.58 g kg^{-1} of total phosphate (P), 9.96 g kg^{-1} of total potassium, 18.82 mg kg^{-1} of available P, 8.54 mg kg^{-1} of NH_4-N, and 12.59 mg kg^{-1} of NO_3-N. BA was not detected in the soil.

2.2. Experiment 1: BA degradation in the soil

The experiment was arranged in a completely randomized design with three replicates that had the following treatments: soil amended with 1.2 μmol g^{-1} soil BA and control soil (CK) amended with distilled water. The soil was maintained at room temperature for at least 48 h prior to treatment with BA. A 12-mL aqueous BA solution (20 mmol L^{-1}) was added to 200 g of soil (dry weight equivalent) and mixed. Soil treated with distilled water served as the control. Soil moisture was adjusted to 40% of the maximum water holding capacity with distilled water. Ten grams of soil (dry weight equivalent) were placed into a 100 mL glass bottle and then incubated at 25 °C in the dark. Water loss was compensated for by adding distilled water daily. Bottles without soil were used as blanks.

Soils were sampled at 0, 24, 36, 48, 72 and 120 h of incubation and then stored at −25 °C until the determination of soil BA concentration. Four hours prior to gas sampling, a 100-mL syringe was used to refresh the air in the headspace of the bottles, which were then hermetically sealed with a rubber plug. The CO_2 production rate was monitored by analysing CO_2 concentrations in the headspace of the bottles.

2.3. Experiment 2: microbial response to BA

To examine the potential effects of BA and to detect the specific taxa responding to BA amendment on soil bacterial and fungal communities, BA was artificially supplied at two levels based on preliminary experiments and work by other researchers (Zhou and Wu, 2012). The experiment was arranged in a completely randomized design with three replicates. A 3-mL aqueous solution of 10 mmol L^{-1} or 20 mmol L^{-1} BA was added to 50 g of soil (dry weight equivalent) and mixed to obtain a low-level BA treatment (0.6 μmol g^{-1} soil, L) and a high-level BA treatment (1.2 μmol g^{-1} soil, H). Soil moisture was maintained at 40% of the maximum water holding capacity. Soil with an equal volume of distilled water was used as the control. Bottles were sealed with breathable sealing membranes and were incubated at 25 °C in the dark. Water loss was compensated for by adding distilled water daily. Soils were sampled on days 2 and 7 and stored at −25 °C and were used for microbiological analyses.

2.4. Experiment 3: response of peanut root rot pathogen Fusarium sp. to BA

The experiment was arranged in a completely randomized design with four replicates. Peanut root rot pathogen *Fusarium* sp. ACCC36194 was obtained from the Agricultural Culture Collection of China (ACCC), Chinese Academy of Agricultural Science. The fungus was grown on potato dextrose agar (PDA) media at 28 °C for 7 days in the dark. The mycelium was scraped and washed twice with 10 mL of sterile water and then filtered through a four-layer sterile lens paper. The filtrate collected was used as the stock conidial and adjusted to 2×10^3 conidia/mL in sterile deionized water using a haemocytometer.

To evaluate the mycelial growth and sporulation capacity, 15 μL of stock conidial suspension was inoculated on the centre of PDA plates amended with BA of various concentrations (0 mmol L^{-1}, CK; 0.1 mmol L^{-1}, B1; and 0.6 mmol L^{-1}, B2) and incubated at 28 °C for 7 days in the dark, with four replicates per treatment.

To determine the conidial germination, 0.1 mL of diluted conidial suspension (2×10^3 conidia mL^{-1}) was spread uniformly on PDA plates containing various concentrations of BA (0 mmol L^{-1}, CK; 0.1 mmol L^{-1}, B1; 0.6 mmol L^{-1}, B2). The plates were incubated at 28 °C for 3 days in the dark.

2.5. CO_2 measurements

CO_2 concentrations were determined using gas chromatography (Agilent 7890A, United States) fitted with a flame ionization detector (FID) under the conditions described by Ma et al. (2015). Briefly, CO_2 was separated using a 2 m stainless steel column with an inner diameter of 2 mm that was packed with 60–80 mesh Porapak Q; CO_2 was then reduced to CH_4 using H_2 in a nickel catalytic converter at 375 °C, and CH_4 was detected by FID at 250 °C. The CO_2 production rate was calculated as previously described by Allison et al. (2009).

2.6. BA extraction and measurements

BA was extracted using a procedure described previously by Dalton et al. (1987). Briefly, two grams of soil (dry weight equivalent) were shaken in 25 mL of 2 mol L^{-1} NaOH for 24 h (180 rpm at 25 °C in the dark). After centrifugation at 6000 *g* for 15 min, the supernatant was acidified to pH 2.5 with 5 mol L^{-1} HCl and mixed with 100 mL of ethyl acetate. The organic phase was collected and evaporated at 40 °C. The residue was dissolved in 2 mL of methanol and filtered (0.22 μm).

The samples were analysed using a high-performance liquid chromatography (HPLC) (Agilent 1200, Germany) with an XDB-C18 column (4.6 mm × 250 mm). The mobile phase was a mixture of methyl cyanide and acetic acid (pH = 2.8) (45:55, v/v). The flow velocity was 0.4 mL min^{-1}. The column temperature was maintained at 40 °C. The UV detector wavelength was set at 230 nm.

2.7. Mycelial growth, sporulation capacity and conidial germination

Mycelial growth was determined by measuring the colony diameter. Then, the sporulation capacity of *Fusarium* sp. was assessed by collecting and counting spores following the procedure described above (Wu et al., 2008, 2015). Spore germination was assessed by determining the colony forming units (CFUs) of the spores (Wu et al., 2008, 2015).

2.8. DNA extraction and quantitative PCR (qPCR)

Total genomic DNA was extracted from 500 mg of freeze-dried soils using a FastDNA spin kit (MP Biomedicals, United States) according to the manufacturer's instructions. The quality and quantity of DNA were analysed using a Nanodrop ND-1000 UV–vis Spectrophotometer (NanoDrop Technologies, United States). The DNA was stored at −25 °C for the subsequent analyses.

Real-time quantitative PCR (qPCR) of the 16S rRNA and ITS rRNA genes was performed in triplicate to assay the bacterial and fungal abundances using a CFX96 Optical Real-time Detection System (Bio-Rad, United States). Primer sets 338F/518R (Muyzer et al., 1993) and ITS1F/ITS4 (Manter and Vivanco, 2007) were used. The reactions were conducted in a 20 μL mixture containing 10 μL SYBR Premix Ex Taq (Takara, China), 0.2 μL of each primer (10 μmol L^{-1}), 1 μL of ten-fold diluted DNA, and 8.6 μL of double-distilled water (ddH_2O). The PCR conditions were performed as described by Zhou and Wu (2012) with some modifications: (i) for the bacterial 16S rRNA gene: 95 °C for 30 s; 94 °C for 30 s, 56 °C for 30 s, and 72 ° C for 30 s for 40 total cycles; (ii) for the fungal ITS sequence: 95 °C for 30 s; 94 °C for 30 s, 57.5 °C for 30 s, and 72 °C for 45 s for 40 total cycles. Melting curve analysis was performed at the end of the PCR run to evaluate the specificity of the amplification. Standard curves were created using 10-fold serial dilutions (10^2–10^8) of a plasmid containing a copy of the 16S or ITS sequence and the 5.8S sequence.

2.9. Bacterial and fungal community compositions through MiSeq sequencing

Fungi are reported to be inconspicuous and slow growing (Rajala et al., 2010). Similar to previous work, the soil fungal community showed no notable change between day 2 and day 7 when assayed using denaturing gradient gel electrophoresis (DGGE) (Fig. S1). However, bacteria respond more rapidly to exogenous organic compounds than fungi. The CO_2 production rate reached its peak at 36 h, and approximately half of the BA in the soil was metabolized by day 2 (Fig. 1). Therefore, soils sampled on days 2 and 7 were used for bacterial and fungal community analysis.

For bacteria, the PCR amplifications were conducted using the 515f/806r primers that targeted the V4 region of the 16S rRNA gene as described by Caporaso et al. (2011). For fungi, ITS1 was amplified with the ITS1F/ITS2 primers as described by Mueller et al. (2014). The reverse primer contained a 6-bp error-correcting barcode unique to each sample. Sequencing was conducted on an Illumina MiSeq platform.

Pairs of reads from the original DNA fragments were merged using FLASH (V1.2.7) (Magoč and Salzberg, 2011) and then assigned to each sample according to the unique barcode of each sample. Raw reads were filtered using the QIIME software package (V1.8.0) as described previously by Bokulich et al. (2013). For bacteria,

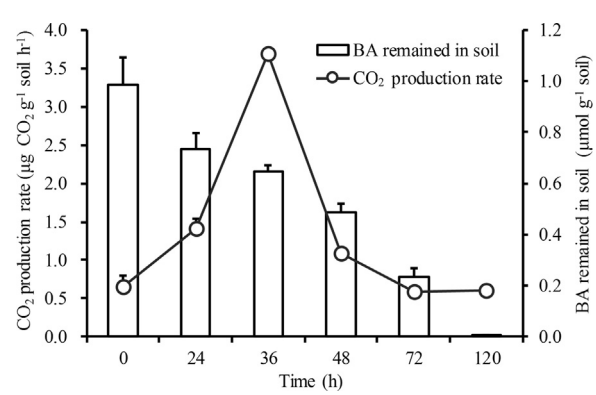

Fig. 1. Dynamics of CO_2 production and residual BA concentration estimated by GC and HPLC during the entire incubation period for soil amended with 1.2 μmol g^{-1} soil BA. Error bars represent the standard deviation between replicates.

sequences were then assigned to operational taxonomic units (OTUs) at 97% similarity by using the UPARSE pipeline (Edgar, 2013). Finally, we picked a representative sequence for each OTU and used the RDP classifier to assign taxonomic data to the corresponding representative sequence (Wang et al., 2007). For fungi, ITS1 reads were clustered using the 'pick_open_reference_otus.py' script with a threshold of 97% identity. The OTU sequences were classified according to the UNITE databases available on the QIIME website (Kõljalg et al., 2013).

2.10. 16S rRNA gene fragment from the Burkholderia species

The primer set of Burk3/BurkR was used to amplify the 16S rRNA genes of the *Burkholderia* species (Salles et al., 2002). The first PCR was performed with the Burk3 primer and the universal primer R1387. The products were diluted 1:1000 and used as the template in the second PCR, which was performed with the primer set Burk3/BurkR. The PCR conditions were 95 °C for 4 min, 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s for 30 total cycles. Three independent PCR products for the control or high-level BA treatment were mixed and purified by using a TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 4.0. The products were cloned into a pEASY-T1 cloning vector (TransGen Biotech, China) using *Escherichia coli* DH5α for the transformation following the manufacturer's protocol. Twenty-five white ampicillin-resistant clones were randomly selected and sent for sequencing (Realgene Biotech, China). Finally, two clone libraries were constructed and 45 recombinant DNA clones (20 for the control, 25 for the high-level BA treatment) were obtained.

Mothur software (v.1.34.4) was used to assign the sequences to operational taxonomic units (OTUs, 97% similarity), and one representative sequence for each OTU was then used for phylogenetic analysis. A phylogenetic tree was constructed using the neighbour-joining method (Saitou and Nei, 1987) as implemented in MEGA 6.06; a bootstrap analysis was performed with 1000 replicates. All sequencing reads generated in this study have been deposited in the GenBank database under accession numbers KT956004-KT956048.

2.11. Statistical analysis

Statistical analyses were conducted using SPSS 16.0 for Windows. Data were expressed as the mean and standard deviation (SD), and different letters indicate significant differences between the results of the samples. Differences in the relative abundances of taxonomic units between samples were tested using Fisher's least significant difference (LSD) test ($P < 0.05$). Using the data of the most abundant bacterial communities at the family level (>0.50%), a heat map and UPGMA clustering were performed using QIIME to depict differences in the composition and structure of the bacterial communities between the treatments.

3. Results

3.1. BA degradation in the soil

CO_2 emission rates were enhanced by adding BA to the soil from 0.66 to 3.70 μg CO_2 g^{-1} soil h^{-1} and increased by approximately 5.6-fold after 36 h of exposure (Fig. 1). Emission rates then gradually decreased to approximately 0.59 μg CO_2 g^{-1} soil h^{-1} on the fourth day. The concentration of BA retained in the soil showed a rapid decrease and declined to 49.18% of the initial concentration within 48 h of BA addition. After a 5-day incubation, the quantity BA recovered from the soil was only 0.007 μmol g^{-1} soil.

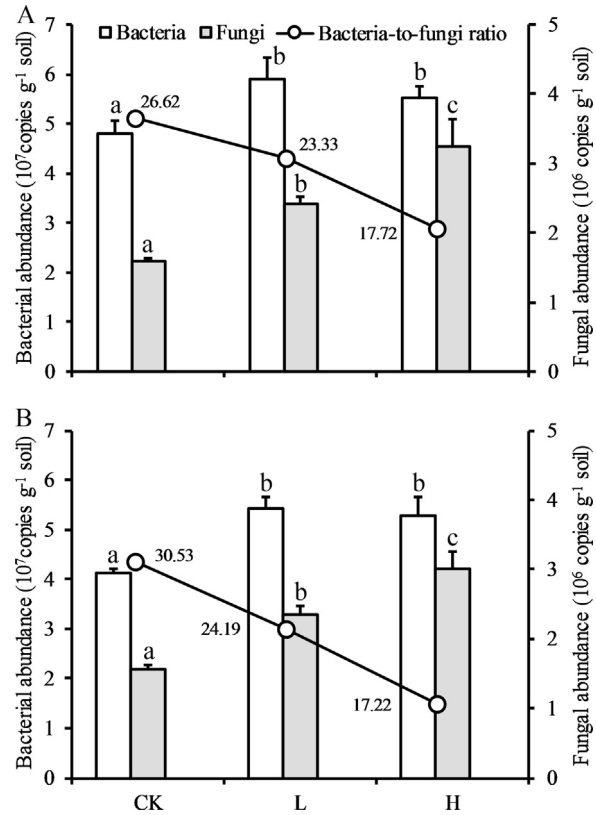

Fig. 2. Effects of BA amendment on soil bacterial and fungal abundances estimated by qPCR and the bacteria-to-fungi ratio on day 2 (A) and day 7 (B). Error bars represent the standard deviation between replicates. Numbers around the '◯' are the ratio of bacteria to fungi. *CK* is the control treatment, *L* is the low BA treatment (0.6 μmol g^{-1} soil), and *H* is the high BA treatment (1.2 μmol g^{-1} soil). Different letters among treatments indicate significant differences ($P < 0.05$).

3.2. Bacterial and fungal abundances in the soil

The bacterial and fungal abundances in the soil were estimated using qPCR. The coefficient of determination of the standard curve was 0.993 for both bacteria and fungi, and the efficiency was 103.8% and 94.4% for bacteria and fungi, respectively. Bacterial and fungal populations were significantly higher in the presence of exogenous BA (Fig. 2). Bacterial abundance (16S rRNA gene copies) on day 2 in the low and high BA treatments increased by 1.31- and 1.28-fold, respectively, compared with the control treatment. For fungal abundance, ITS rRNA gene copies increased consistently with the increase in BA concentration. Similar results were observed on day 7. The bacteria-to-fungi ratio underwent a steady decline as the concentration of BA increased.

3.3. Response of the fungal community composition to BA amendment

An average of 43315 ± 17114 fungal sequence reads per sample was obtained (Table S1). The quantity of OTUs was reduced in the high BA treatment. At the phylum level, the fungal community was dominated by members of *Ascomycota* and *Basidiomycota*, representing approximately 81.47% to 85.21% of the total classified fungal sequences (Fig. 3). The relative abundance of *Ascomycota* was significantly increased in the low-level BA treatment, whereas the *Zygomycota* abundance was reduced under the same condition. The *Chytridiomycota* community was markedly inhibited in the presence of BA.

At the genus level, fungal OTUs showing a significant change are listed in Table 1. The abundances of *Arxula* and *Metarhizium* in the soil incubated with BA were markedly reduced, whereas the

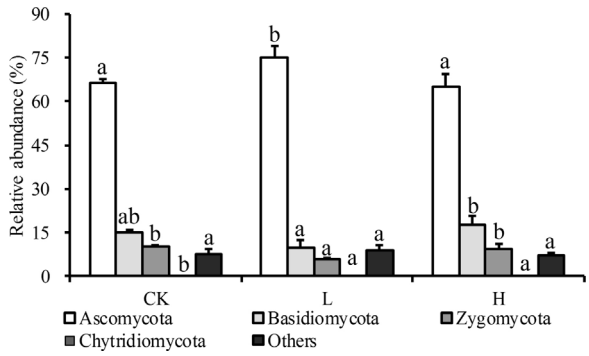

Fig. 3. The average distribution of the fungal community at the phylum level in the soil based on ITS1 sequence analysis. Error bars represent the standard deviation between replicates. CK is the control treatment, L is the low BA treatment (0.6 μmol g^{-1} soil), and H is the high BA treatment (1.2 μmol g^{-1} soil). Different letters among treatments indicate significant differences ($P < 0.05$).

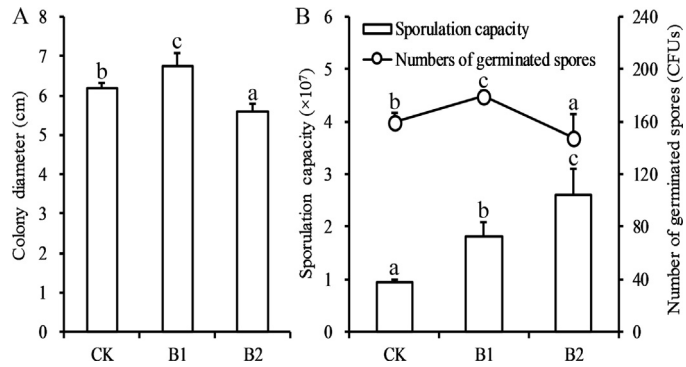

Fig. 4. Effects of BA amendment on *Fusarium* sp. growth (A) and sporulation capacity and conidial germination (B). Error bars represent the standard deviation between replicates. *CK* is the control treatment, *B1* and *B2* are the BA treatments of 0.1 and 0.6 mmol L^{-1}, respectively. Different letters among treatments indicate significant differences ($P < 0.05$).

abundances of *Trichoderma* and *Fusarium* were notably enhanced in response to BA amendment. The abundances of *Bionectria*, *Cystofilobasidium* and *Rickenella* were markedly increased in the high BA treatment while an unidentified genus belonging to *Nectriaceae* was markedly increased in the low BA treatment.

3.4. Response of the peanut root rot pathogen Fusarium sp. to BA amendment in vitro

The growth of peanut root rot pathogen *Fusarium* sp. was significantly promoted by the low BA treatment (0.1 mmol L^{-1}), but was reduced by the concentration of 0.6 mmol L^{-1}. Similar results were observed regarding the number of germinated spores. Sporulation capacity was significantly enhanced as the concentration of BA increased (Fig. 4).

3.5. Changes in bacterial community composition

For bacteria, an average of 39414 ± 15448 sequence reads per sample was acquired (Table S1). There was no difference in the number of OTUs between the treatments. At the phylum level, more than 97% of the qualified reads were assigned to 29 phyla in all soil samples. Overall, the dominant phyla across all samples were *Chloroflexi* (26.74%–30.20%), *Acidobacteria* (15.80%–17.13%), *Actinobacteria* (10.55%–13.44%), *Alphaproteobacteria* (9.21%–10.48%), *Betaproteobacteria* (3.11%–13.75%), *AD3* (6.48%–8.11%) and *Gammaproteobacteria* (2.09%–2.96%) (Fig. 5). Compared with the control, the relative abundance of *Betaproteobacteria* was increased by approximately 3.53- and 4.42-fold in the soil amended with low- or high-level BA, respectively, whereas the other dominant phyla such as *Actinobacteria*, *AD3* and *Gammaproteobacteria* gradually declined with the addition of BA.

Because most bacterial genera have yet to be described, an analysis was performed at the family level (Fig. 6). Although bacterial families, such as *Rhodospirillaceae*, *Solibacteraceae*, *Sinobacteraceae*, *Sphingomonadaceae*, *Gaiellaceae*, *Nocardioidaceae* and *Syntrophobacteraceae*, responded positively or negatively with the increasing BA concentration, differences did not reach statistical significance (Table S2). The relative abundance of *Thermogemmatisporaceae* and *FFCH4570* significantly decreased with the addition of BA compared with the control. Three families belonging to the *Betaproteobacteria* were detected, but only *Burkholderiaceae* showed a significant increase (by 8.79- and 10.96-fold). Deep taxonomic analyses revealed that the genus *Burkholderia* was the most responsive to BA incubation (Fig. 7). The relative abundance of *Burkholderia* increased from 0.71% in the control soil to 7.47% and 10.12% in the soil amended with low- or high-level BA, respectively.

3.6. Shifts in the Burkholderia species in response to BA amendment

Because *Burkholderia* was significantly enhanced by BA amendment, clone libraries targeting *Burkholderia* 16S rRNA gene

Table 1
Fungal genera exhibiting significant changes in the soil treatments.

OTU number	Phylum	Family	Genus	Relative abundance (%)		
				CK	L	H
OTU_11	*Ascomycota*	*Nectriaceae*	*Fusarium*	2.55 ± 0.02a	4.10 ± 0.62b	4.68 ± 0.81b
OTU_20	*Ascomycota*	*Bionectriaceae*	*Bionectria*	1.38 ± 0.02a	1.22 ± 0.31a	2.37 ± 0.44b
OTU_134	*Ascomycota*	*Nectriaceae*	*unidentified*	10.11 ± 1.00a	18.93 ± 0.21b	12.83 ± 2.30a
OTU_10	*Ascomycota*	*Clavicipitaceae*	*Metarhizium*	4.32 ± 1.25b	2.31 ± 0.39a	1.87 ± 0.47a
OTU_28	*Ascomycota*	*Hypocreaceae*	*Trichoderma*	0.18 ± 0.12a	1.83 ± 0.62c	0.90 ± 0.07b
OTU_23	*Ascomycota*	*Pleosporaceae*	*Alternaria*	0.54 ± 0.10b	0.70 ± 0.12b	0.26 ± 0.02a
OTU_4	*Ascomycota*	*Trichomonascaceae*	*Arxula*	8.02 ± 4.59b	0.82 ± 0.66a	0.98 ± 0.06a
OTU_2	*Basidiomycota*	*unidentified*	*unidentified*	10.21 ± 0.09b	6.79 ± 0.39a	12.38 ± 1.67b
OTU_27	*Basidiomycota*	*Marasmiaceae*	*unidentified*	2.83 ± 1.04ab	3.43 ± 1.97b	0.12 ± 0.20a
OTU_223	*Basidiomycota*	*unidentified*	*unidentified*	1.10 ± 0.22b	0.53 ± 0.24a	1.35 ± 0.17b
OTU_51	*Basidiomycota*	*Cystofilobasidiaceae*	*Cystofilobasidium*	0.29 ± 0.06a	0.30 ± 0.05a	0.56 ± 0.21b
OTU_54	*Basidiomycota*	*Incertae sedis*	*Rickenella*	0.02 ± 0.02a	1.02 ± 0.51ab	1.55 ± 0.82b
OTU_13	*Zygomycota*	*Mortierellaceae*	*Mortierella*	2.32 ± 0.65b	1.24 ± 0.26a	2.36 ± 0.50b
OTU_18	*Zygomycota*	*unidentified*	*unidentified*	1.89 ± 0.21b	0.95 ± 0.08a	1.22 ± 0.40a
OTU_9	*Zygomycota*	*unidentified*	*unidentified*	5.30 ± 0.27b	3.19 ± 0.41a	5.61 ± 0.82b

CK is the control treatment, *L* is the low BA treatment (0.6 μmol g^{-1} soil), and *H* is the high BA treatment (1.2 μmol g^{-1} soil). Numbers with different letters in each line indicate a significant difference between treatments ($P < 0.05$).

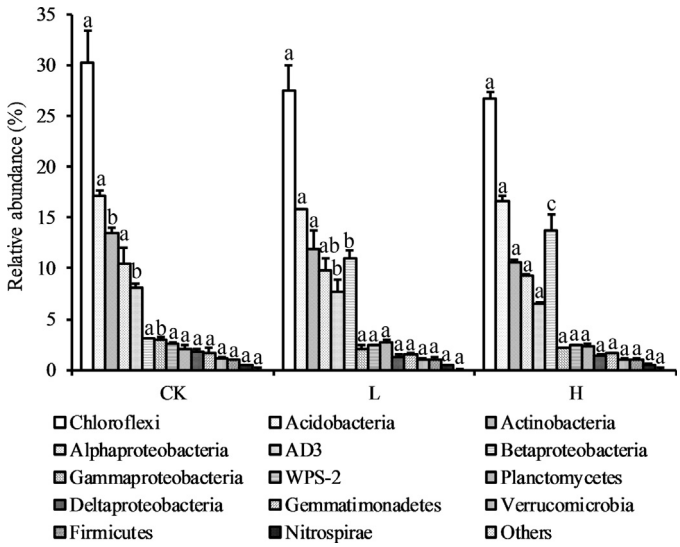

Fig. 5. The average distribution of the bacterial community at the phylum level in the soil based on 16S rRNA gene analysis. CK is the control treatment, L is the low BA treatment (0.6 μmol g^{-1} soil), and H is the high BA treatment (1.2 μmol g^{-1} soil). Error bars represent the standard deviation between replicates. Different letters among treatments indicate significant differences ($P < 0.05$).

fragments (length 510 bp) were constructed to assess the change in the composition of *Burkholderia* species. Clones obtained from the control and BA treatments were assigned to 9 OTUs at 97% sequence identity. To analyse the distribution of sequences within the genus *Burkholderia*, a phylogenetic tree was generated using the representative sequences of each OTU and 21 reference sequences downloaded from the NCBI (Fig. 8). BA amendment primarily influenced the relative abundance of OTU1 (from 30% to 52%), OTU2 (from 5% to 28%), OTU3 (from 25% to 8%) and OTU4 (from 30% to 0%). OTU1, OTU3 and OTU4 are closely related to *B. caledonica*, *B. soli* and *B. caryophylli*, respectively. OTU2 is closely related to *B. diffusa* and is grouped with the *B. cepacia* complex (BCC).

4. Discussion

The persistence of phenolic acids in the soil determines whether the chemicals accumulate to phytotoxic concentrations. The two primary fractions of phenolic acids in the soil, the dissolved and the reversibly adsorbed fractions, are thought to be associated with allelopathic interactions (Blum, 1998; Cecchi et al., 2004). However, they are easily utilized by soil microbes, resulting in their short half-lives in soils (Zhou and Wu, 2012, 2013). Our present study found that BA concentrations declined sharply once added to the soil and were reduced from 0.99 to 0.007 μmol g^{-1} soil within a 5-day period. This result was substantiated by our determination of the CO_2 production rate, suggesting that several soil microbes in the soil could grow using BA as a carbon source. Soil microbes are ubiquitous, perform important functions linked to soil fertility, and are closely correlated with aboveground plant performance (Jousset et al., 2014). We found that BA amendment of the soil significantly increased both fungal and bacterial abundances and reduced the ratio of bacteria to fungi, which is consistent with previous observations in the rhizosphere of cucumbers supplied continuously with *p*-coumaric acid (Zhou and Wu, 2012).

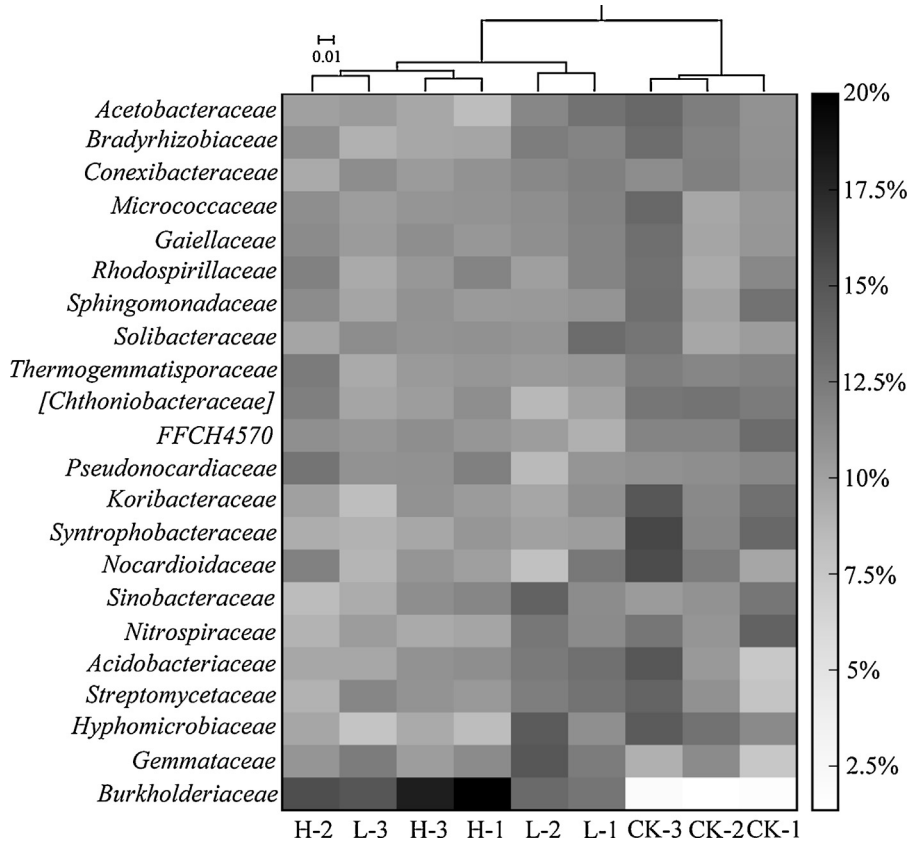

Fig. 6. Heat map of dominant bacterial families in the soil incubated with and without BA. The colour code indicates relative abundance, ranging from white (low abundance) to black (high abundance). *CK* is the control treatment, *L* is the low BA treatment (0.6 μmol g^{-1} soil), and *H* is the high BA treatment (1.2 μmol g^{-1} soil).

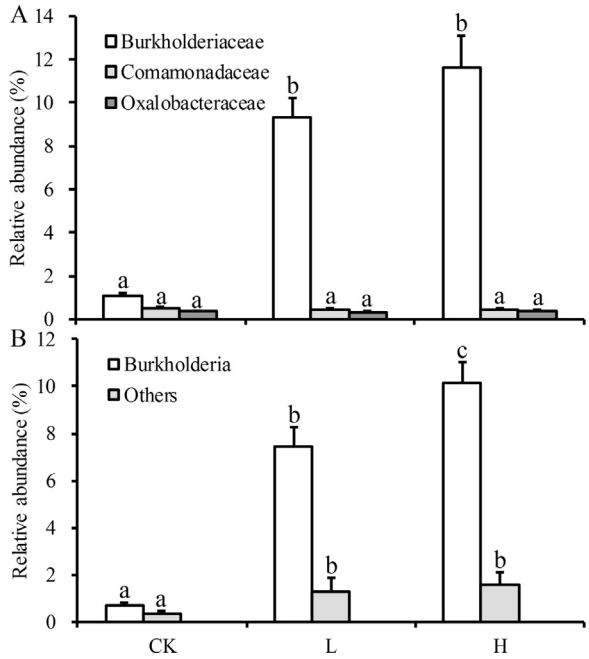

Fig. 7. Dominant families belonging to *Betaproteobacteria* (A) and the composition of the family *Burkholderiaceae* (B). *CK* is the control treatment, *L* is the low BA treatment (0.6 μmol g^{-1} soil), and *H* is the high BA treatment (1.2 μmol g^{-1} soil). Different letters among treatments indicate significant differences ($P < 0.05$).

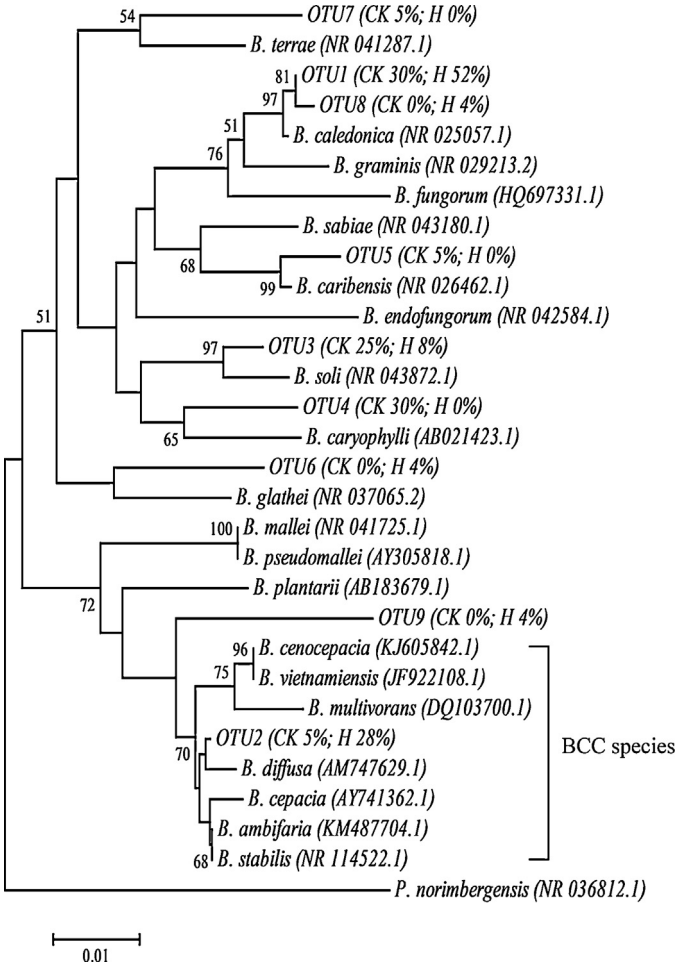

Fig. 8. Phylogenetic tree of partial 16S rRNA genes of *Burkholderia* species in the soil treated with and without BA. The tree was constructed by using the neighbour-joining method, and the sequence of *Pandoraea norimbergensis* was used as an out-group. A bootstrap analysis was performed with 500 replications; only values above 50 are shown. *CK* is the control and *H* is the high BA treatment. The number following indicates the proportion of the total 16S rRNA genes in each treatment. *BCC* represents the *B. cepacia* complex. The scale bar represents the proportion of nucleotide substitutions.

Ascomycota is one dominant fungal phylum in the soil, which includes many plant pathogens and is considered a major source of toxins (Ecker et al., 2005; Li et al., 2014b). A relatively higher level of *Ascomycota* has been observed in soils under long-term monoculture (Chen et al., 2012; Li et al., 2014b). Further analysis in this study showed that *Bionectria* and *Fusarium*, belonging to the *Ascomycota*, increased significantly under BA treatment, whereas *Metarhizium* and *Arxula* were decreased. *Metarhizium* is a genus of entomopathogenic fungi, frequently isolated from the soil. They parasitize a broad range of insect species from numerous orders and are used as biological control agents (Bischoff et al., 2009). Species of *Arxula* can assimilate and ferment many compounds as carbon sources and are very attractive for biotechnological applications (Kunze and Kunze, 1994). Several phytopathogens have been described in *Bionectria* and *Fusarium*; *Fusarium*, in particular, harbours notorious pathogenic plant fungi with a wide variety of hosts and infection strategies (Li et al., 2014b; Michielse and Rep, 2009). *Fusarium oxysporum* f. sp. *lycopersici* degrades phenolic compounds *via* the β-ketoadipate pathway required for its pathogenicity (Michielse et al., 2012).

Peanut root rot caused by *Fusarium* spp. is considered to be the primary cause of poor performance in continuous peanut production in red soil (Wang and Chen, 2005). Therefore, the significant increase of *Fusarium* in response to BA amendment indicates that the presence of soilborne pathogens induced by particular components of peanut root exudates might contribute to the increased incidence of soilborne diseases (*e.g.* root rot disease) in the soil of peanut monoculture. The *in vitro* experiment also showed that BA significantly promoted the mycelial growth, sporulation capacity and conidial germination of the peanut root rot pathogen *Fusarium* sp. This is also supported by previous research showing that cinnamic acid promoted the incidence of Fusarium wilt in cucumber (Ye et al., 2004). However, OTUs closely related to *Trichoderma,* known for their biocontrol activities against many fungal and bacterial diseases, including *Fusarium* spp., had a positive response to the BA amendment (Dubey et al., 2007; Vinale et al., 2008). Thus, our results indicate that the net effects of phenolic acid on the soil fungal community cannot be simply characterized as positive or negative and that complicated interactions among the induced microbial species should also be considered.

For bacteria, BA amendment increased the relative abundance of the phylum *Betaproteobacteria* but reduced the abundance of the phylum *Actinobacteria. Betaproteobacteria* is reported to be more abundant in the rhizosphere of diseased tomatoes than in healthy plants (Li et al., 2014c). Members of *Actinobacteria* are known to be antibiotic producers and showed a positive correlation with soil suppressiveness (Cretoiu et al., 2013; Li et al., 2014c). Therefore, BA amendment may lead to modifications in soil suppressiveness to soilborne pathogens. Among the soil bacterial taxa, the genus *Burkholderia*, belonging to *Betaproteobacteria*, was significantly promoted in the soil amended with BA. This might be related to their impressive capacity for aromatic compound catabolism (Pumphrey and Madsen, 2008; Suárez-Moreno et al., 2012).

The *Burkholderia* species, isolated from a wide range of niches, is divided into two groups by phylogenetic analysis: human, animal and plant pathogens (*B. glumae*, *B. pseudomallei* and *B. mallei*, as well as the 17 defined species of the BCC) and potentially beneficial plant-associated species (Sène et al., 2000; Suárez-Moreno et al.,

2012). In this study, BA amendment increased the burkholderial species closely related to *B. caledonica*, whereas species closely related to *B. soli* and *B. caryophylli* were reduced. *B. caledonica* and *B. caryophylli* have been reported to have 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, which is involved in the lowering of plant ethylene levels (Shaharoona et al., 2007; Suárez-Moreno et al., 2012). BA amendment also increased the proportion of burkholderial species closely related to BCC groups. Several species such as *B. cenocepacia* and *B. plantarii* are reported to be plant pathogens (Lee and Chan, 2007). BCC bacteria can also inhibit many soilborne pathogens and promote crop growth (Holmes et al., 1998). Therefore, further work is needed to elucidate how BCC bacteria interact with other organisms.

Plant health and fitness greatly depend on interactions with soil microorganisms (Chen et al., 2012, 2014; Kelderer et al., 2012; Li et al., 2014b; Mazzola and Manici, 2012). A diseased crop is the result of complex interactions between the plant, the pathogens, and the other microbial populations within the soil (Berendsen et al., 2012; Xu et al., 2012). Therefore, the presence of pathogenic microbes alone cannot fully explain the prevalence of soilborne diseases, and other soil microbial groups should be taken into consideration. We have shown that not only was the abundance of the potential pathogens *Bionectria* sp. and *Fusarium* sp. induced by BA amendment, but other specific microbes such as *Trichoderma* and *Metarhizium* were also affected; reduction of the actinobacterial community and stimulation of the BCC group were significantly associated with BA amendment. Therefore, our findings support the hypothesis that soilborne disease is a complex, community-based phenomenon rather than a population-level mechanism based solely on the presence of individual pathogens or microorganisms (Berendsen et al., 2012; Minz et al., 2013). This suggests that the interactions among specific soil microbes induced by phenolic acids constitute an important influential force on the soilborne diseases (*e.g.* root rot disease) resistance of peanut plants under monoculture conditions.

5. Conclusions

When added to the soil, BA cannot persist for a long period; its effects on the soil were interpreted by mediating the microbial community. BA amendment significantly increased the abundance of both bacteria and fungi and stimulated or inhibited specific bacterial and fungal species. Particular genera, such as *Fusarium*, *Bionectria*, *Trichoderma*, *Metarhizium* and *Burkholderia*, were stimulated. *Fusarium* and *Bionectria* contain many plant pathogens, whereas *Trichoderma* has many strains with biological control activities. *Metarhizium* is a genus of entomopathogenic fungi. *Burkholderia*, which is functionally a remarkably diverse genus, contains both plant growth promoting strains and pathogens. Under field condition, BA can be continuously produced and secreted into the surrounding soil by roots. This could reinforce the effects of the microbial utilization of BA in the rhizosphere. Interactions between bacteria and plant pathogens are increasingly recognized to affect plant community composition and performance; these results, therefore, enhance our understanding of how phenolic acids influence these interactions and consequently plant health.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (41671306, 41371290), the GanPo 555 Talents Program of Jiangxi Province, China.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.11.001.

References

Allison, S.D., LeBauer, D.S., Ofrecio, M.R., Reyes, R., Ta, A.M., Tran, T.M., 2009. Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. Soil Biol. Biochem. 41, 293–302.
Baziramakenga, R., Simard, R.R., Leroux, G.D., 1994. Effects of benzoic and cinnamic acids on growth, mineral composition, and chlorophyll content of soybean. J. Chem. Ecol. 20, 2821–2833.
Berendsen, R.L., Pieterse, C.M., Bakker, P.A., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486.
Bever, J.D., Platt, T.G., Morton, E.R., 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu. Rev. Microbiol. 66, 265–283.
Bhowmik, P.C., 2004. Sorption of benzoic acid onto soil colloids and its implications for allelopathy studies. Biol. Fert. Soils 40, 345–348.
Bischoff, J.F., Rehner, S.A., Humber, R.A., 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101, 512–530.
Blum, U., 1998. Effects of microbial utilization of phenolic acids and their phenolic acid breakdown products on allelopathic interactions. J. Chem. Ecol. 24, 685–708.
Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D. A., Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.
Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108 (Suppl. 1), 4516–4522.
Cecchi, A.M., Koskinen, W.C., Cheng, H.H., Haider, K., 2004. Sorption-desorption of phenolic acids as affected by soil properties. Biol. Fert. Soils 39, 235–242.
Chen, M.N., Li, X., Yang, Q.L., Chi, X.Y., Pan, L.J., Chen, N., Yang, Z., Wang, T., Wang, M., Yu, S., 2012. Soil eukaryotic microorganism succession as affected by continuous cropping of peanut-pathogenic and beneficial fungi were selected. PLoS One 7, e40659.
Chen, M.N., Li, X., Yang, Q.L., Chi, X.Y., Pan, L.J., Chen, N., Yang, Z., Wang, T., Wang, M., Yu, S., 2014. Dynamic succession of soil bacterial community during continuous cropping of peanut (*Arachis hypogaea* L.). PLoS One 9, e101355.
Cretoiu, M.S., Korthals, G.W., Visser, J.H.M., van Elsas, J.D., 2013. Chitin amendment increases soil suppressiveness toward plant pathogens and modulates the actinobacterial and oxalobacteraceal communities in an experimental agricultural field. Appl. Environ. Microbiol. 79, 5291–5301.
Dalton, B.R., Weed, S.B., Blum, U., 1987. Plant phenolic acids in soils: a comparison of extraction procedures. Soil Sci. Soc. Am. J. 51, 1515–1521.
Dubey, S.C., Suresh, M., Singh, B., 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. Biol. Control 40, 118–127.
Ecker, D.J., Sampath, R., Willett, P., Wyatt, J.R., Samant, V., Massire, C., Hall, T.A., Hari, K., McNeil, J.A., Büchen-Osmond, C., Budowle, B., 2005. The Microbial Rosetta Stone Database: a compilation of global and emerging infectious microorganisms and bioterrorist threat agents. BMC Microbiol. 5, 19.
Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–998.
Holmes, A., Govan, J., Goldstein, R., 1998. Agricultural use of *Burkholderia* (*Pseudomonas*) *cepacia*: a threat to human health? Emerg. Infect. Dis. 4, 221–227.
Huang, L.F., Song, L.X., Xia, X.J., Mao, W.H., Shi, K., Zhou, Y.H., Yu, J.Q., 2013. Plant-soil feedbacks and soil sickness: from mechanisms to application in agriculture. J. Chem. Ecol. 39, 232–242.
Inderjit Saini, M., Kaur, H., 2005. Experimental complexities in evaluating the comparative phytotoxicity of chemicals with different modes of action. Environ. Exp. Bot. 53, 97–104.
Jordan, D.L., Bailey, J.E., Barnes, J.S., Bogle, C.R., Bullen, S.G., Brown, A.B., Edmistena, K.L., Dunphy, E.J., Johnson, P.D., 2002. Yield and economic return of ten peanut-based cropping systems. Agron. J. 94, 1289–1294.
Jousset, A., Becker, J., Chatterjee, S., Karlovsky, P., Scheu, S., Eisenhauer, N., 2014. Biodiversity and species identity shape the antifungal activity of bacterial communities. Ecology 95, 1184–1190.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Oldmaa, K.P., Saag, L., Saar, R., Schüssler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Kelderer, M., Manici, L.M., Caputo, F., Thalheimer, M., 2012. Planting in the 'inter-row' to overcome replant disease in apple orchards: a study on the effectiveness of the practice based on microbial indicators. Plant Soil 357, 381–393.

Kunze, G., Kunze, I., 1994. Characterization of *Arxula adeninivorans* strains from different habitats. Antonie van Leeuwenhoek 65, 29–34.
Lanoue, A., Burlat, V., Henkes, G.J., Koch, I., Schurr, U., Röse, U.S., 2010. De novo biosynthesis of defense root exudates in response to Fusarium attack in barley. New Phytol. 185, 577–588.
Lee, Y.A., Chan, C.W., 2007. Molecular typing and presence of genetic markers among strains of banana finger-tip rot pathogen, *Burkholderia cenocepacia*, in Taiwan. Phytopathology 97, 195–201.
Li, P.D., Wang, X.X., Li, Y.L., Wang, H.W., Liang, F.Y., Dai, C.C., 2010. The contents of phenolic acids in continuous cropping peanut and their allelopathy. Acta Ecol. Sin. 30, 2128–2134 (in Chinese).
Li, P.D., Dai, C.C., Wang, X.X., Zhang, T.L., Chen, Y., 2012. Variation of soil enzyme activities and microbial community structure in peanut monocropping system in subtropical China. Afr. J. Agric. Res. 7, 1870–1879.
Li, X.G., Zhang, T.L., Wang, X.X., Hua, K., Zhao, L., Han, Z.M., 2013. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen. Int. J. Biol. Sci. 9, 164–173.
Li, X.G., Ding, C.F., Hua, K., Zhang, T.L., Zhang, Y.N., Zhao, L., Yang, Y.R., Liu, J.G., Wang, X.X., 2014a. Soil sickness of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to direct allelopathy. Soil Biol. Biochem. 78, 149–159.
Li, X.G., Ding, C.F., Zhang, T.L., Wang, X.X., 2014b. Fungal pathogen accumulation at the expense of plant-beneficial fungi as a consequence of consecutive peanut monoculturing. Soil Biol. Biochem. 72, 11–18.
Li, J.G., Ren, G.D., Jia, Z.J., Dong, Y.H., 2014c. Composition and activity of rhizosphere microbial communities associated with healthy and diseased greenhouse tomatoes. Plant Soil 380, 337–347.
Ling, N., Zhang, W.W., Wang, D.S., Mao, J.G., Huang, Q.W., Guo, S.W., Shen, Q.R., 2013. Root exudates from grafted-root watermelon showed a certain contribution in inhibiting *Fusarium oxysporum* f sp. *niveum*. PLoS One 8, e63383.
Liu, W.X., Wang, Q.L., Wang, B.Z., Wang, X.B., Franks, A.E., Teng, Y., Li, Z.G., Luo, Y.M., 2015. Changes in the abundance and structure of bacterial communities under long-term fertilization treatments in a peanut monocropping system. Plant Soil 395, 415–427.
Ma, L., Shan, J., Yan, X.Y., 2015. Nitrite behavior accounts for the nitrous oxide peaks following fertilization in a fluvo-aquic soil. Biol. Fert. Soils 51, 563–572.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Mandal, S.M., Chakraborty, D., Dey, S., 2010. Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal. Behav. 5, 359–368.
Manter, D.K., Vivanco, J.M., 2007. Use of the ITS primers, ITS1F and ITS4, to characterize fungal abundance and diversity in mixed-template samples by qPCR and length heterogeneity analysis. J. Microbiol. Methods 71, 7–14.
Mazzola, M., Manici, L.M., 2012. Apple replant disease: role of microbial ecology in cause and control. Annu. Rev. Phytopathol. 50, 45–65.
Michielse, C.B., Rep, M., 2009. Pathogen profile update: *Fusarium oxysporum*. Mol. Plant Pathol. 10, 311–324.
Michielse, C.B., Reijnen, L., Olivain, C., Alabouvette, C., Rep, M., 2012. Degradation of aromatic compounds through the β-ketoadipate pathway is required for pathogenicity of the tomato wilt pathogen *Fusarium oxysporum* f. sp. *lycopersici*. Mol. Plant Pathol. 13, 1089–1100.
Minz, D., Ofek, M., Hadar, Y., 2013. Plant rhizosphere microbial communities. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, pp. 56–84.
Mueller, R.C., Paula, F.S., Mirza, B.S., Rodrigues, J.L.M., Nüsslein, K., Bohannan, B.J.M., 2014. Links between plant and fungal communities across a deforestation chronosequence in the Amazon rainforest. ISME J. 8, 1548–1550.
Muyzer, G., de Waal, E., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain. Appl. Environ. Microbiol. 59, 695–700.
Pumphrey, G.M., Madsen, E.L., 2008. Field-based stable isotope probing reveals the identities of benzoic acid-metabolizing microorganisms and their in situ growth in agricultural soil. Appl. Environ. Microbiol. 74, 4111–4118.
Rajala, T., Peltoniemi, M., Hantula, J., Mäkipää, R., Pennanen, T., 2010. RNA reveals a succession of active fungi during the decay of Norway spruce logs. Fungal Ecol. 4, 437–448.
Sène, M., Doré, T., Pellisier, F., 2000. Effect of phenolic acids in soil under and between rows of a prior sorghum (*Sorghum bicolor*) crop on germination, emergence and seedling growth of peanut (*Arachis hypogea*). J. Chem. Ecol. 26, 625–637.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Salles, J.F., De Souza, F.A., van Elsas, J.D., 2002. Molecular method to assess the diversity of *Burkholderia* species in environmental samples. Appl. Environ. Microbiol. 68, 1595–1603.
Shaharoona, B., Jamro, G.M., Zahir, Z.A., Arshad, M., Memon, K.S., 2007. Effectiveness of various *Pseudomonas* spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.). J. Microbiol. Biotechnol. 17, 1300–1307.
Singh, H.P., Batish, D.R., Kohli, R.K., 1999. Autotoxicity: concept, organisms, and ecological significance. Crit. Rev. Plant Sci. 18, 757–772.
Suárez-Moreno, Z.R., Caballero-Mellado, J., Coutinho, B.G., Mendonça-Previato, L., James, E.K., Venturi, V., 2012. Common features of environmental and potentially beneficial plant-associated *Burkholderia*. Microb. Ecol. 63, 249–266.
Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L., Lorito, M., 2008. Trichoderma-plant-pathogen interactions. Soil Biol. Biochem. 40, 1–10.
Wang, M.Z., Chen, X.N., 2005. Obstacle and countermeasure of sustainable high yield for peanut in low-hilly red soil region. J. Peanut Sci. 34, 17–22 (in Chinese).
Wang, Q.G., Garrity, M., Tiedje, J.M., Cole, J.R., 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. 73, 5261–5267.
Wang, B.B., Li, R., Ruan, Y.Z., Ou, Y.N., Zhao, Y., Shen, Q.R., 2015. Pineapple-banana rotation reduced the amount of *Fusarium oxysporum* more than maize-banana rotation mainly through modulating fungal communities. Soil Biol. Biochem. 86, 77–86.
Wu, H.S., Raza, W., Fan, J.Q., Sun, Y.G., Bao, W., Shen, Q.R., 2008. Cinnamic acid inhibits growth but stimulates production of pathogenesis factors by in vitro cultures of *Fusarium oxysporum* f sp. *niveum*. J. Agric. Food Chem. 56, 1316–1321.
Wu, Z.J., Yang, L., Wang, R.Y., Zhang, Y.B., Shang, Q.H., Wang, L., Ren, Q., Xie, Z.K., 2015. In vitro study of the growth, development and pathogenicity responses of *Fusarium oxysporum* to phthalic acid, an autotoxin from Lanzhou lily. World J. Microbiol. Biotechnol. 31, 1227–1234.
Xu, L.H., Ravnskov, S., Larsen, J., Nilsson, R.H., Nicolaisen, M., 2012. Soil fungal community structure along a soil health gradient in pea fields examined using deep amplicon sequencing. Soil Biol. Biochem. 46, 26–32.
Ye, S.F., Yu, J.Q., Peng, Y.H., Zheng, J.H., Zou, L.Y., 2004. Incidence of Fusarium wilt in *Cucumis sativus* L. is promoted by cinnamic acid: an autotoxin in root exudates. Plant Soil 263, 143–150.
Zhao, Y.P., Wu, L.K., Chu, L.X., Yang, Y.Q., Li, Z.F., Azeem, S., Zhang, Z.X., Fang, C.X., Lin, W.X., 2015. Interaction of *Pseudostellaria heterophylla* with *Fusarium oxysporum* f. sp. *heterophylla* mediated by its root exudates in a consecutive monoculture system. Sci. Rep. 5, 8197.
Zhou, X.G., Wu, F.Z., 2012. *p*-Coumaric acid influenced cucumber rhizosphere soil microbial communities and the growth of *Fusarium oxysporum* f sp. *cucumerinum* Owen. PLoS One 7, e48288.
Zhou, X.G., Wu, F.Z., 2013. Artificially applied vanillic acid changed soil microbial communities in the rhizosphere of cucumber (*Cucumis sativus* L.). Can. J. Soil Sci. 93, 13–21.
Zhou, X.G., Gao, D.M., Liu, J., Qiao, P.L., Zhou, X.L., Lu, H.B., Wu, X., Liu, D., Jin, X., Wu, F. Z., 2014. Changes in rhizosphere soil microbial communities in a continuously monocropped cucumber (*Cucumis sativus* L.) system. Eur. J. Soil Biol. 60, 1–8.
van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J., Wardle, D.A., 2013. Plant-soil feedbacks: the past, the present and future challenges. J. Ecol. 101, 265–276.