Biogas slurry application alters soil properties, reshapes the soil microbial community, and alleviates root rot of *Panax notoginseng*

Chengxian Wang [1,2,*], Jianfeng Liu [1,2,3,*], Changmei Wang [1,2,3], Xingling Zhao [1,2,3], Kai Wu [1,2,3], Bin Yang [1,2,4], Fang Yin [1,2,3], Wudi Zhang [1,2,3]

[1] Yunnan Research Center of Biogas Technology and Engineering, School of Energy and Environment Science, Yunnan Normal University, Kunming 650500, PR China

[2] Engineering and Research Center of Sustainable Development and Utilization of Bioenergy, Ministry of Education, Yunnan Normal University, Kunming 650500, PR China

[3] Jilin Dongsheng Institute of Biomass Energy Engineering, Tonghua 134118, PR China

[4] Graduate School, Yunnan Normal University, Kunming 650500, PR China

[*] These authors contributed equally to this work.

Corresponding author: Wudi Zhang

Email address: 2071@ynnu.edu.cn

Abstract

Background. *Panax notoginseng* is, an important herbal medicine in China, where this crop is cultivated by replanting of seedlings. Root rot disease primarily by threatens the sustainability of *P. notoginseng* cultivation. Water flooding (WF) is widely used can control numerous soilborne diseases and biogas slurry (BS) has positive effects on the soil physiochemical properties and microbial community structure and has the potentials to suppress soilborne pathogens. Hence, BSF may be an effective way to alleviate root rot disease of *P. notoginseng*, and the underlying mechanism needs to be elucidated.

Methods. In this study, we conducted a microcosm experiment to determine if BSF can reduce the abundance of pathogens in soil and alleviate root rot of *P. notoginseng*. Microcosms containing soil collected from a patch of *P. notoginseng* showing symptoms of root rot disease, in which the soil was subjected to WF or BSF at two concentrations for two durations (15 and 30 days), followed by investigating changes in the physicochemical properties, and an assay to estimate culturable microorganisms and root rot ratio. Subsequently, we compared changes in the microbial community structure of soils under BSF with changes in WF and untreated soils through bacterial 16S rRNA (16S) and fungal internal transcribed spacer (ITS) genes amplicon high-throughput sequencing.

Results. WF treatment did not significantly change the soil microbiota. Conversely, BSF treatments altered the physicochemical properties and reshaped the bacterial and fungal community, reduced the relative abundance of potential fungal pathogens (*Fusarium, Cylindrocarpon, Alternaria,* and *Phoma*) and suppressed the culturable fungi and *Fusarium*. These changes in microbial community structure corresponded to , eventually decreased the root rot ratios. The mechanisms of fungal pathogen suppression by BSF involved several factors, including the presence of more anaerobic/conductive conditions, altered soil physicochemical properties, enriched anaerobic and culturable bacteria, and higher phylogenetic relatedness in the bacterial community. Overall, BSF , all of which promoted the bacterial community and e suppressed the fungal and pathogenic taxa.

Conclusions. BS application can reshape the soil microbial community, reduce the abundance of potential pathogens, and alleviate root rot of *P. notoginseng*. Hence, BSF is a promising biological practice for controlling root rot disease in *P.* notoginseng

Commented [LMG1]: I assume, please check

Commented [LMG2R1]:

production.

Keywords Biogas slurry; *Panax notoginseng*; Root rot disease; Soil physicochemical properties; Microbiota;

Introduction

Biogas production, by anaerobic digestion of human and animal waste, straw, and other organic material, has emerged as a potentially important source of renewable energy (Kougias & Angelidaki 2018). In recent decades, the number of biogas plants in China and other countries has increased significantly (Kougias & Angelidaki 2018). Rapid development of biogas production has increased in biogas residue, including a large amount of biogas slurry (BS). BS is a high-quality organic fertilizer with significant residual organic carbon, available nitrogen, and other minerals for crop planting (Abubaker et al. 2012; Insam et al. 2015). Hence, application of BS changes the structure of soil microbial community, increases the soil microbial diversity and activity, improves~~has been reported to improve~~ soil quality, and enhances crop yield (Abubaker et al. 2012; Cristina et al. 2020; Walsh et al. 2012). Furthermore, BS contains numerous organic compounds (such as volatile fatty acid) and a high concentration of ammonia, which could suppress *Fusarium* spp. and other soil pathogens~~, partly accountingaccount for its biological activity~~ (Cao et al. 2014; Huang et al. 2015b).~~-~~ Consistent with other organic amendments, BS application can ~~significantly~~ suppress~~es~~ soilborne pathogens and reduce the incidence of plant diseases (Cao et al. 2016; Insam et al. 2015). For example, Cao et al. (2016) showed that the application of BS, under both normal moisture and flooding conditions (BSF),~~,~~ ~~could~~ suppressed *Fusarium* wilt disease of watermelons~~, with the latter treatment being more effective~~. Appropriate application of BS to soil contributes to the decreased use of pesticides and chemical fertilizers, making BS a suitable alternative with positive effects in terms of sustainable agriculture production and environmental protection (Insam et al. 2015; Walsh et al. 2012).~~–~~

Panax notoginseng (Burk.) F. H. Chen (“Sanqi” in Chinese), is a member of the family Araliaceae, ~~is~~ and one of the most important herbal medicines in China. At present, Wenshan County in the Yunnan Province (Southwest China) is the geo-authentic habitat for Sanqi planting. Due to the specific ecological habitat of Sanqi and

Commented [LMG3]: Enclose phrases in commas

Formatted: Font color: Red

~~the~~ use of a continuous monoculture system, replanting failure is highly prevalent. This has led to the planting area being extended to the surrounding areas and beyond, which further negatively affects the yield and quality of Sanqi and leads to more serious diseases, such as mildew and root rot. ~~effects of~~ ~~a~~Abiotic and biotic factors, including deterioration of soil physicochemical characteristics, nutrient imbalance, soilborne diseases, and accumulation of phytotoxic allelochemical substrates cause replanting failure (Liu et al. 2019; Wu et al. 2008). Root rot is the foremost fungal disease associated with soil-borne pathogens, such as *Fusarium*, *Alternaria*, *Cylindrocarpon*, and *Phoma* (Li et al. 2020; Miao et al. 2006; Wang et al. 2021). Of these, *Fusarium* spp. are the primary pathogens due to their wide range of hosts and strong tolerance to stressful conditions (e.g., drought and high temperatures), making them difficult to control (Liu et al. 2019). Different measures have been explored to mitigate diseases in the Sanqi production system, among which, rotation (with maize, rape, and wheat) is a preferred practice (Liu et al. 2019; Tang et al. 2020). However, even after 10–20 years, rotation cannot completely eliminate diseases because of the long lifespan of pathogens (Tang et al. 2020). Sanqi cultivation still faces replant failure due to sterilization of soil using fungicides and fumigation (Yang et al. 2015). In addition, fungicides are not environmentally benign and are gradually becoming unavailable owing to strict regulation of agrochemicals. Hence, it has become increasingly urgent to develop more effective and non-chemical biological measures, such as soil flooding and/or addition of organic materials (straw, BS). Water flooding (WF), an ancient and widely used practice in China and other Asian countries, ~~is~~ effectively ~~for~~ controlls numerous soilborne diseases (Niem et al. 2013). BSF, which incorporates organic materials and associated microorganisms, can increase soil microbial activity, and promote~~s~~ nutrient availability (Cao et al. 2016; ~~(~~Dahunsi et al. 2021). ~~In turn, it~~BSF also has positive effects on the soil physiochemical properties (such as increase of soil pH, contents of AK, NH_4^+-N, water-soluble carbon, and water-soluble nitrogen) and microbial community structure (suppression of~~, with the potential to suppress~~ soilborne pathogens), with potential to alleviate plant disease (Cao et al. 2014; Cao et al. 2016). Meanwhile, the practice of BSF, introducing organic materials into soil (Abubaker et al. 2012; Insam et al. 2015) and rapidly creating reductive/anaerobic conditions (Cao et al. 2016), is similar to anaerobic soil disinfestation (ASD) by flooding soil after addition of organic residues (Blok et al. 2000). Since ASD has been widely used due to effective disinfestation of various soilborne pathogens (Hewavitharana & Mazzola 2016; Strauss

Formatted: Font color: Red

Formatted: Font color: Red

& Kluepfel 2015; Zhou et al. 2019), BSF may have similar application prospect.

Regarding Sanqi root rot disease, studies investigating changes in the soil physiochemical properties and microbial community under BSF and/or WF treatments are lacking, particularly for changes in pathogen abundance. We hypothesized that, compared with WF, BSF was more effective to suppress pathogen and alleviate root rot disease of *P. notoginseng*. Here, we conducted a microcosm experiment using Sanqi root rot soil, which was treated with WF and two concentrations of BSF. We aimed to (1) explore changes in ~~the~~ physicochemical properties and ~~the~~ composition of microbial community in the treated soils, (2) assess ~~the~~ efficacy of WF and BSF to suppress the pathogens and alleviate root rot symptoms, and (3) elucidate the mechanisms underlying this response.

Formatted: Font color: Red

Formatted: Font color: Red

Materials and Methods

Biogas slurry and soil characteristics

BS was collected from an internal-circulation biogas reactor, using vegetable juice waste as ~~the~~ input material, at ~~the~~ Bio-energy and Environment Engineering Research Group, Yunnan Normal University, Kunming, China. The reactor had been stably operated for 6 months. Chemical~~The chemical~~ characteristics of the BS were as follows: total solid 1.0 ± 0.2%, chemical oxygen demand 7072 ± 65 mg/L, total N 612.2 ± 22.4 mg/L, NH_4^+-N 282.7 ± 14.6 mg/L, NO_3^--N 49.6 ± 5.3 mg/L, and pH 6.5 ± 0.2. Bulk soil samples were collected from a Sanqi plantation in Wenshan (23°40′N, 102°35′E, 1400 m alt.), Yunnan Province, China, in January 2019. This site is classified as Latosols based on the Chinese soil taxonomy. The region is characterized by a subtropical climate, with a mean annual precipitation of 1100–1319 mm and a mean annual temperature of 15–18 °C. Sanqi was consecutively cultivated for 5 years and had suffered severe root rot disease at this site. *Fusarium* spp. have been frequently isolated and identified as the main pathogens underlying this disease. Chemical characteristics of the initial soil were as follows: pH 6.8 ± 0.2, organic matter 19.5 ± 1.7 g/kg, total N 925.5 ± 43.2 mg/kg, available P 66.0 ± 4.5 mg/kg, and available K 94.4 ± 7.1 mg/kg. Approximately 10 kg of the continuous-cropping soil was randomly collected from the 0–20 cm soil layer, homogeneously mixed, sieved through a 2-mm mesh to remove stones and plant debris.

Experimental design and soil sampling

A soil microcosm experiment was conducted to investigate the effects of WF and BSF treatments on soil physicochemical properties and microbiota. Three treatments, each with three replicates, were performed in 500-mL microcosms (glass bottles) as follows: (1) water flooding treatment (CK): 250 g soil flooded with 250 mL sterilized water; (2) diluted-BS treatment (CH): 250 g soil flooded with 250 mL diluted 50%-concentration BS (equivalent to ~ 0.3 g N/kg soil); (3) original BS treatment (CF): 250 g soil flooded with 250 mL original BS (equivalent to ~ 0.6 g N/kg soil). Specifically, after adding BS at two concentrations to the initial soil (untreated soil, named "Soil"), homogeneously mixed slurry samples were immediately collected (named "CH0d" and "CF0d" respectively,", representing two BS-flooded soils without incubation), then the glass bottles were sealed and incubated in the dark at 28 °C. The water flooding treatment was performed with ~~the~~ a similar procedure.

After incubation for 15 and 30 days, day 15 (CK15d, CH15d, and CF15d, representing water and two BS-flooded soils with 15 day's incubation) and day 30 soil samples (CK30d, CH30d, and CF30d, representing water and two BS-flooded soils with 30 day's incubation) were collected (~ 20 g per sample).~~.~~ Thus, a total of 27 samples were obtained. Each sample was divided into two parts: one part was used to analyze the soil physicochemical properties and assess the number of culturable bacteria and fungi, and the other was stored at –80 °C for subsequent DNA extraction.

Analysis of soil physicochemical properties

Soil pH and oxidation-reduction potential (Eh) were determined using a PHS-3C Meter with the corresponding electrodes (INESA Scientific Instrument Co., Ltd, Shanghai, China) and a 1:2.5 soil/water (w/v) suspension. Electrical conductivity (EC) was measured with a 1:5 soil/water (w/v) suspension using a DDS-11A Conductivity Meter (INESA Scientific Instrument Co., Ltd). Ammonia nitrogen was measured by a continuous flow analyzer (FIAstar TM 5000 System; FOSS, Hilleroed, Denmark). Potential toxic organic acids (mainly volatile fatty acids [VFAs], including acetate, propionate, butyrate, and valerate) were analyzed using gas chromatography on a GC-9790II (FULI Apparatus Co. Ltd., Shanghai, China), as previously described (Zhao et al. 2018). All ~~the~~ values were obtained from three replicates in each treatment.

Assay of culturable Bacteria, Fungi, and *Fusarium*

Commented [LMG4]: Revise text to include rebuttal comments about why 1:1 soil/water ratio. "A 1:1 soil:water ratio was selected following (**Wen et al. (2015)**.

Commented [LMG5]: Address the effect of sealing mesocosms on oxygen availability in Discussion

To assess the effects of water or BS treatments on the microbial community in the soils, we determined the population densities of culturable bacteria, fungi, and *Fusarium* (one of the potential pathogens) using a standard 10-fold dilution plating assay. Briefly, ~~the~~ bacterial population was enumerated on nutrient agar (NA) medium plates, and colony forming units (CFUs) were counted after incubation at 30 °C for 2 days. Fungi and *Fusarium* were enumerated using Martin's rose bengal agar (RBM) and Komada's selective medium (Komada 1975), respectively, and both were counted after incubation at 25 °C for 5 days. All ~~the~~ values were obtained from three replicates.

Pathogenicity assay of soil on Sanqi root

A pathogenicity assay (potential to cause root rot) of the water or BS-treated soils above was performed on Sanqi roots *in vitro* according to a modified-previous method (Luo et al. 2019). Briefly, on the 30th day of the experiment, surface water was removed and the remaining treated soils were air-dried for 4 days (about 40% water holding capacity) and then thoroughly mixed. Afterwards, healthy 1-year-old roots were washed with sterile water, then surface sterilized with 1% sodium hypochlorite for 6 min, and finally washed 4 times with sterile water. The roots were transferred to a plastic container containing the treated soils and were completely covered with the soils. Each treatment (CK, CH, and CF) and the untreated soil contained three replicates (each with 10 roots). All treatments were randomly placed in an incubator at 25 °C and watered every 3 days to keep the water holding capacity at approximately 40%. After 30 days, ~~the~~ root rot ratios (%) were calculated as the number of roots showing rot-rooted symptoms divided by the total number of tested roots.

Soil DNA extraction and sequencing

Total genomic DNA was extracted from each sample using a PowerSoil® DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). ~~The~~ DNA concentration and quantity were evaluated using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), after which the extracted DNA was stored at –20 °C until use. Marker genes, amplified by polymerase chain reaction (PCR), were sequenced to characterize the community composition and diversity of bacteria and fungi. Prokaryotic~~The prokaryotic~~ 16S V3-V4 and fungal ITS2 regions were amplified using the primer pairs 341F (CCTAYGGGRBGCASCAG)/ 806R (GGACTACNNGGGTATCTAAT) and ITS3-

Formatted: Font: Italic

2024F(GCATCGATGAAGAACGCAGC)/ ITS4-2409R (TCCTCCGCTTATTGATATGC), respectively (Takahashi et al. 2014; Toju et al. 2012). ~~The~~ PCR was performed using a Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). The amplification conditions were as follows: initial denaturation at 95 °C for 2 min, followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. Subsequently, the PCR products were purified with a GeneJET™ Gel Extraction Kit (Thermo Fisher Scientific) and used to construct sequencing libraries using an Ion Plus Fragment Library Kit (Thermo Fisher Scientific), following the manufacturer's recommendations. Library quality was assessed using a Qubit@ 2.0 Fluorometer (Thermo Fisher Scientific). Finally, the library was sequenced on an Ion S5™ XL platform and 600-base pair single-end reads were generated. The sequencing data generated was deposited in the NCBI Sequence Read Archive database (accession numbers PRJNA661430 and PRJNA661668 for the bacterial and fungal sequences, respectively).

Bioinformatics analyses

~~The r~~Raw sequencing reads were filtered and analyzed using the QIIME software (v1.9.1) (Caporaso et al. ~~,~~2010). Briefly, ~~the~~ primer sequences and low quality read with scores below Q30 were filtered, and chimeras were detected using Chimera UCHIME. Filtered high-quality sequences with ≥ 97% similarity were clustered and assigned to the same operational taxonomic unit (OTU) using Uparse software (7.0.1001) (Edgar 2013). To obtain taxonomic information on the bacterial and fungal OTUs, a representative sequence of each OTU was generated and aligned against the Silva (v132) and Unite (v7.2) databases, respectively. OTU abundance was normalized using a standard sequence number corresponding to each sample with the least sequences (45,000 sequences for bacteria and 32,000 for fungi).

Alpha diversity indices, including observed-species and Shannon, were applied to analyze within-sample diversity. Beta diversity analysis was used to evaluate differences between samples (treatments). Principal coordinate analysis (PCoA) and hierarchical cluster analysis were both based on the Bray-Curtis distance using an OTU abundance table, and then visualized by "ggplot2" (3.3.3) and "ggtree" (Yu et al. 2017) in R software (v3.5.1), respectively. Linear discriminant analysis (LDA) coupled with effect size was performed using LEfSe software (Segata et al. 2011), and the default

LDA score was 3.5. Metastat analysis in R software was performed using permutation tests between groups at the genus level to obtain *P* values (adjusted with the "false discovery rate" method).

Predictions of bacterial and fungal community functions were performed using FAPROTAX (Louca et al. 2016) and FunGuild (Nguyen et al. 2016) software, respectively. The nearest taxon index (NTI) was calculated for the microbial phylogenetic diversity using the null model independent swap with 999 randomization runs and 1000 iterations, utilizing ~~the~~ "Picante" package (Stegen et al. 2012) in R software.

Statistical analysis

To determine the significance of differences in ~~the~~ microbial community composition between groups, non-parametric multivariate analysis of variance (PERMANOVA, transformed data by Bray-Curtis, permutation = 999) was performed with the adonis function of ~~the~~ "vegan" package (v ##) in R ~~software~~. Analysis of Spearman's correlations among microbial genera, environmental factors, putative pathogens, and the most abundant bacteria/fungi were performed in with base functions in R version ##. A partial Mantel (999 permutations) was then applied to calculate the correlation between environmental factors and the microbial ~~community using the "vegan" package in R software~~ v##. Regression analyses were performed to investigate the relationships between the root rot ratio and biotic/abiotic factors (culturable microorganisms, soil properties, and alpha/beta-diversity index). Statistical differences between treatments (soil properties, alpha diversity, NTI values) were calculated using a one-way analysis of variance followed by a Tukey's HSD test ($P < 0.05$ being considered significant~~.~~).

Commented [LMG6]: Provide package version

Commented [LMG7]: Package version

Results

Physicochemical properties of soil

To evaluate changes in the physicochemical properties of continuous-cropping soil under WF and BSF treatments, VFAs, pH, Eh, EC, and NH_4^+-N were determined (Fig. 1). ~~Results~~~~The results demonstrated that the a~~Addition of BS (CH0d-CF0d) ~~resulted in incorporation of higher contents of~~increased acetate and propionate and lowered contents of butyrate and valerate in the initial soils ("Soil")~~,~~. ~~with t~~Total VFAs were

significantly ~~increase~~ higher in BS-addition groups (t-test with $P < 0.05$: CH0d 282±10 vs. Soil 7±1; CF0d 582±82 vs. Soil 7±1).~~(CH0d and CF0d).~~ The levels of acetate and propionate gradually decreased to below the limits of detection (CH30d and CF30d) after 30 days, while butyrate levels increased and valerate was maintained at nearly constant levels.

Following WF and BSF treatment for 15 or 30 days, pH and NH_4^+-N increased while Eh decreased as compared to initial soil (CK15d or CK30 vs. Soil; CH15d or CH30 vs. Soil; CF15d or CF30 vs. Soil).~~,~~ This was most notable in the BSF treatments, which induced more conductive/anaerobic conditions (Eh < 0 mV) with BS treatment compared with those under WF treatment. Additionally, compared with those in the corresponding CK, pH and EC were higher, whereas Eh was lower in the BSF-treated soils. This trend was more clearly visible at higher concentrations of BS (CF treatment) and after a longer treatment duration (Eh value: -68 mV in CH15d, -105.33 mV in CF15d; -88 mV in CH30d, -112 mV in CF30d).

Pathogenicity assay of soil

Pathogenicity (i.e. potential to cause root rot) of the water or BS-treated soils on Sanqi roots was assayed, and root rot ratio was calculated to evaluate the effect of different treatment. Typically, symptoms of root rot included soft, necrotic, and brown roots (Fig. 2a), while health root showed no these symptoms and kept intact (Fig. 2b). Results~~The pathogenicity assay~~ indicated that both untreated continuous-cropping ("Soil") and water-flooded ("CK") soil showed the highest pathogenicity potential, as approximately 90% of the root showed root rot symptoms (Fig. 2c). Conversely, BSF treatment (CH and CF) significantly decreased ($P < 0.05$) the root rot ratio to as low as 10–20% (Fig. 2c).

Population of culturable microorganisms in soil

As shown in Fig. 2d, compared with ~~that in~~ the untreated soil ("Soil"),~~,~~ 15 days of BSF treatment suppressed the population of culturable microorganisms, with a significant decrease in the fungal and *Fusarium* populations ($P < 0.05$). After 30 days, the fungal and *Fusarium* population further decreased slightly while the bacterial population was restored to its original level. On the 30th day,~~ the~~ number of fungi was

Commented [LMG8]: Use reasonable and consistent significant figures

Formatted: Font: Font color: Red

Formatted: Font: Font color: Red

the least in CF, and the smallest *Fusarium* population was observed in CH. Moreover, the ratios of *Fusarium*/fungi and bacteria/fungi were both significantly reduced by BSF treatment, with ~~the~~ CH treatment showing the lowest *Fusarium*/fungi ratio. In contrast, compared with the untreated soil, water-flooded treatment (CK) showed no obvious effects on the culturable microorganisms tested ($P < 0.05$).

Formatted: Font color: Red

Alpha diversity of the microbial community

Next, we evaluated the effects of WF and BSF treatments on the microbial community based on the bacterial 16S and fungal ITS marker gene sequencing. After quality control, the sequences were clustered into 4984 and 1123 OTUs for bacteria and fungi, respectively. Alpha diversity analysis showed that both bacterial and fungal observed-species/Shannon indices demonstrated similar trends after BSF (CH15d, CF15d, CH30d, and CF30d) and WF (CK15d and CK30d) treatment (Table 1). Specifically, Shannon indices of both CH0d and CF0d decreased significantly compared with those in the original soil ($P < 0.05$). After incubation for 15 or 30 days, there was a notable increase in both indices in BSF-treated soils compared to CH0d (or CF0d), although the values were lower than their corresponding CK values at the same duration. Additionally, both indices of diluted-BSF (CH, CH15d and ~~/~~CH30d) were slightly higher than those of original-BSF (CF, CF15d and CF30d) at the same treatment duration.

Structure of microbial community

PCoA and hierarchical cluster analysis were applied to compare the dissimilarities and hierarchical clustering of the microbial community between treatments, respectively. For bacteria, PERMANOVA analysis showed a significant difference among all groups ($R^2 = 0.89$, $P = 0.001$), and PCoA ordination demonstrated that the first two axes accounted for 81~~.06~~% of the variation (Fig. 3a). All samples were mainly separated into three clusters: "~~the~~ Soil~~ /CK15.30d (Soil,~~ CK15d-CK30d" and "CH0d-CF0d" samples were separately clustered; the remaining BSF-treated samples (CH15d, CH30d, CF15d, and CF30d) formed the third cluster, where "CH15d-CH30d" (CH, diluted-BS treated samples) and "CF15d-CF30d" (CF, initial BS-treated samples) were slightly separated along the first axis, consistent with the hierarchical cluster analysis (Fig. 3c). A similar clustering pattern was found for fungi (PERMANOVA, $R^2 = 0.81$, $P = 0.001$), except BSF-treated samples were more dispersed than the corresponding

bacteria samples (Fig. 3b, d). Based on the different soil treatments and sample-clustering modes, samples were combined to 5 larger groups to perform the comparative analysis in subsequent sections: (1) Soil (representing initial soils); (2) CHCF0d (CH0d and CF0d, representing BS flooded soils without incubation); (3) CK15.30d (CK15d and CK30d, representing water flooded soils with 15 and 30 day's incubation); (4) CH15.30d (CH15d and CH30d, representing 50% diluted-BS flooded soils with 15 and 30 day's incubation); and (5) CF15.30d (CF15d and CF30d, representing original BS flooded soils with 15 and 30 day's incubation). To assess statistical significance of differences between the 5 groups in the bacterial and fungal communities, differences between pairwise groups was examined using PERMANOVA analysis. Results revealed ~~a~~ significant differences between groups, except for "Soil vs. CK15.30d" (Table S1, Bacteria: $R^2 = 0.45$, $P = 0.062$; Fungi: $R^2 = 0.27$, $P = 0.053$).

Composition of ~~the~~ fungal community

All fungal OTUs were classified into 10 phyla and 190 genera. Of those, one OTU (OUT_1, only classified at the kingdom level, Dataset S1) that did not exist in the initial soil was found at a notably high abundance in CH0d (63~~.07~~%) and CF0d (66%) compared to that in the other groups. After incubation for 15 or 30 days, the abundance of OTU_1 declined to 10% in CH15.30d and 14% in CF15.30d. Overall, BSF significantly modulated the composition of fungal community from phylum to genus levels. Specifically, two dominant fungal phyla which could be classified across all samples were *Ascomycota* and *Basidiomycota* (Fig. 4a). *Ascomycota* were highly enriched in ~~the~~ Soil and CK15.30d (67~~.08~~–75~~.14~~%) but were depleted in CH15.30d (11~~.29~~–15~~.49~~%, metastats analysis with $P < 0.05$). At the genus level, six genera that presented average relative abundances above 1% were *Fusarium*, *Chaetomium*, *Staphylotrichum*, *Setophoma*, *Humicola*, and *Saitozyma*. Of these, *Fusarium* was dominant across all samples (Fig. 4b) and was significantly depleted in CH15.30d soils compared with that in CK15.30d and CF15.30d soils ($P < 0.05$). The other five genera were significantly decreased after BSF treatment ($P < 0.05$). LEfSe analysis indicated that some *Ascomycota* affiliations (*Sordariales*, *Staphylotrichum*, *Setophoma*, and *Fusarium*) declined markedly in CH15.30d compared with those in CK15.30d soils ($P < 0.05$; Fig. 5f).

Furthermore, comparison of the fungal OTUs between Soil and CHCF0d

Commented [LMG9]: Use reasonable significant figures here and in Figure 3a

Commented [LMG10]: Percentages usually don't have significant figures, unless measurement error was extraordinarily low.

demonstrated that 219 OTUs were common to both groups or unique to CHCF0d (Fig. 5a). According to the published literature (Li et al. 2020; Liu et al. 2019; Miao et al. 2006), combined with the taxa present in our results, four taxa (*Fusarium, Cylindrocarpon, Alternaria, and Phoma*) were detected as potential fungal pathogens of Sanqi root rot. Next, we analyzed changes in the relative abundance of these fungi in response to BSF and/or WF treatments (Fig. 5c). Compared with those in the initial soil, the levels of these four genera decreased by 4- to 200-fold after BSF treatment (CH15.30d and CF15.30d), except for a slight increase in *Fusarium* in the CF15.30d group (21.169% in Soil, 28.429% in CF15.30d). Conversely, there was a slight increase in the abundance of *Fusarium*, *Cylindrocarpon*, and *Phoma* in WF soils (CK15.30d) compared to that in the initial soil.

Composition of bacterial community

Prokaryote OTUs were classified into two kingdoms (Archaea and Bacteria), 64 phyla, and 608 genera. The most abundant Archaea OTU (phylum *Euryarchaeota*; genus *Methanocorpusculum*) was present in CF30 (0.15%). The five dominant phyla, which accounted for 81.84% of the bacterial community across all samples, were *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Synergistetes*, and *Acidobacteria* (Fig. 4c). After BSF treatment, ~~the~~ relative abundance of *Proteobacteria, Actinobacteria,* and *Acidobacteria* decreased (from 4~~65.53~~% to 18.~~17–40.06~~%, 11.~~36~~% to 0.4–1.94%, and from 10.~~07~~% to 0.5–1.9%, respectively), while that of *Firmicutes*, *Synergistetes*, and *Bacteroidetes* increased significantly ($P < 0.05$; from 0.88% to 10.42-16.22%, from 0.04% to 3.29–14.03%, and from 10.83% to 21.75–49.31%, respectively). At the genus level, the top 10 genera, with more than 1% abundance, were *unidentified_Rikenellaceae*, *Trichococcus*, *Macellibacteroides*, *Lactobacillus*, *Arcobacter*, *unidentified_Synergistaceae*, *Proteiniphilum*, *Sphingomonas*, *Pseudomonas*, and *Bacteroides* (Fig. 4d). LEfSe analysis showed that the phyla *Firmicutes* (affiliated order *Clostridiales*; family *Ruminococcaceae*; genus *Anaerovorax*), *Synergistetes* (genus *unidentified_Synergistaceae*), and *Bacteroidetes* (genus *unidentified_Rikenellaceae*, *Macellibacteroides*, and *Proteiniphilum*) were consistently and significantly enriched following BSF treatment ($P < 0.05$). *Actinobacteria* (genus *Bryobacter*), *Proteobacteria* (genus *Sphingobium* and *unidentified_GammaProteobacteria*), and *Gemmatimonadetes* (family *unidentified_Gemmatimonadaceae*) were the marker taxa (identified by LEfSe) for the

Formatted: Font color: Red

initial soil (Fig. 5e). Among the top genera, *unidentified_Rikenellaceae*, *Macellibacteroides*, *Lactobacillus*, *unidentified_Synergistaceae*, *Proteiniphilum*, *Pseudomonas*, and *Sedimentibacter* were specific to the CH0d and CF0d groups compared with the initial soil. Furthermore, abundance of some bacterial genera differed significantly between groups; relative abundance of *Trichococcus*, *Lactobacillus,* and *Arcobacter* was significantly higher in CH0d ~~/~~CF0d than that in other groups ($P < 0.05$). Additionally, there were no significant differences between the initial soil and CK15.30d at either the phylum or genus level (Fig. 4c, d).

Comparing the bacterial OTUs between the initial Soil and CHCF0d soils revealed that 466 OTUs were unique to CHCF0d (Fig. 5b). These OTUs are mainly facultative or anaerobic bacteria (*unidentified_Rikenellaceae, unidentified_Synergistaceae,* and *Sedimentibacter*).

Nearest taxon index analysis of ~~the~~ microbial community

NTI analysis of the bacterial and fungal samples revealed that all NTI values were higher than zero (Fig. 5d), and the average NTI value (3.22 ± 0.78) of the bacterial community was significantly higher ($P < 0.05$) than that of the fungi (1.91 ± 0.58), providing evidence for phylogenetic assembly, especially in bacteria. After BS amendment (CH0d and CF0d), the bacterial NTI decreased significantly compared with that in the initial soil ($P < 0.05$). The bacterial NTI of CH0d increased after 15 days (CH15d) and continued to increase after 30 days (CH30d), while no significant changes were observed under 100% BSF or CK treatment. Furthermore, NTI of the CH treatments was higher than that of the corresponding CF treatments, especially after 30 days. A similar trend was observed for the fungal NTI, except that the NTI of CH30d was lower than that of CH15d.

Function analysis of microbial community

Functions of bacterial OTUs were annotated using FAPROTAX. As shown in (Fig. S1a), all BSF-treated samples harbored enriched functions involved in anaerobic metabolism, such as "methanogenesis," "sulfate_respiration," and "methanotrophy," which was consistent with the increased abundance of anaerobic bacteria (e.g., *Methanosaeta*, and *Desulfovibrio*).

Fungal functional prediction using FunGuild showed that functions related to "Plant Pathogen" were more enriched in "Soil and ~~/~~CK15.30d" samples than in

"CH15.30 and ~~/~~CF15.30d" samples (Fig. S1b).

Correlation between the potential pathogens and top abundant taxa

Spearman's correlation analysis between the four potential pathogens and the top 20 abundant bacteria, as well as fungi at the genus level revealed that the four pathogens were significantly negatively correlated with most bacteria; however, they were significantly positively correlated with most fungi (Fig. 6a, b). In addition, as shown in Fig. 6c, correlations between the most abundant bacteria and fungi revealed a higher proportion of negative (71%) than positive (29%) correlations.

Correlation between ~~the~~ microbial community and soil physicochemical properties

As indigenous microbiota ~~the~~ shape soil condition ~~is largely influenced and shaped by the indigenous microbiota~~, we used Eh, and EC, pH, NH_4^+-N, and VFAs as environmental factors to clarify the relationships between microbiota and soil properties. ~~The~~ A Mantel test showed that environmental factors were significantly correlated with changes in bacteria (r = 0.55, P = 0.001) and fungi (r = 0.42, P = 0.002). In addition, Spearman's rank correlation analysis was used to evaluate the correlations between soil properties and bacteria or fungi at the genus level. ~~As shown in Fig. 6c,~~ Among the top 25 genera, most bacteria were significantly negatively correlated with Eh (P < 0.05), but positively correlated with EC, NH_4^+-N, and VFAs (acetate, propionate, and valerate, Fig. 6c).

Most fungi were significantly negatively correlated with EC, pH, NH_4^+-N, and VFAs (P < 0.05), and positively correlated with Eh. Particularly, the potential pathogens (*Fusarium, Cylindrocarpon,* and *Phoma)* were significantly negatively correlated with EC, pH, NH_4^+-N, and all the tested VFAs (P < 0.05).

Additionally, VPA analysis (Fig. S2) revealed that soil physicochemical properties, treatment modes (BS application or not), and treatment days explained 79% and 69% of the observed variation in bacterial and fungal compositions, respectively. Soil properties, which were clearly affected by treatment mode, explained 20% and 31.~~1~~% of the observed variation in bacterial and fungal compositions, respectively, while treatment days only explained a small portion (2% and 3.~~3~~%, respectively).

Correlation between root rot ratio and biotic/abiotic factors

Commented [LMG11]: Insert reference

Linear regression analyses (Fig. S3) showed that the root rot ratio was significantly ($P < 0.05$) positively correlated with the number of culturable fungi and *Fusarium*, ratio of *Fusarium* to fungi, ratio of fungi to bacteria, alpha-diversity (Shannon index) of the bacterial and fungal communities, and Eh value in soil, but negatively correlated with the number of culturable bacteria ($P = 0.16$), concentrations of total VFAs ($P = 0.056$), EC ($P < 0.05$), and NH_4^+-N ($P < 0.05$). Moreover, the root rot ratio was significantly correlated with the bacterial and fungal beta-diversity index (PCoA1) ($P < 0.05$).

Discussion

Maintaining soil fertility and controlling soilborne diseases are vital for sustainable crop production. BS, an organic fertilizer of high quality and biological toxicity, is an excellent candidate for green agriculture (Cao et al. 2016; Insam et al. 2015; Walsh et al. 2012). This study investigated the impacts of BSF application on the microbial community (including potential pathogens) and occurrence of root rot symptoms in Sanqi continuous soil.

BSF improved soil conditions

BSF has similar characteristics to ASD; ~~by~~both introduce organic materials into soil and creating reductive/anaerobic conditions. Mechanisms underlying the suppression and efficacy of ASD include strong anaerobic/reductive conditions, shifts in microbial population, and production of organic acids and ammonia (Huang et al. 2015b; Momma et al. 2011). ASD was recently adopted to control Sanqi soil disease and alleviate replant failure (Li et al. 2019). Considering the similarities between the procedures, the mechanisms through which ASD suppresses pathogens may also be partly applied to BSF. The present study showed that *Firmicutes*-affiliated anaerobes (*Clostridiales* and *Ruminococcaceae*; frequently enriched in response to ASD treatment), producers of toxic VFAs (Huang et al. 2015a; Momma et al. 2013; Mowlick et al. 2013), were significantly enriched after BSF treatment. High concentrations of VFAs can suppress pathogens in ASD-treated soil (Momma et al. 2006). In the present study, both BS treatments incorporated large amounts of BS-derived VFAs (acetate and propionate) into the initial soils, and persistence of butyrate generated in the soils throughout the experiment showed potential for suppressing pathogens. This was demonstrated by the negative correlation between pathogens and VFAs. Notably, many

Formatted: Font: Font color: Red

facultative and anaerobic taxa (such as *unidentified_Rikenellaceae*, *Trichococcus*, *Macellibacteroides*, and *Lactobacillus*), unique to CHCH0d and derived from BS, were incorporated into the initial soil by BSF. Furthermore, a portion of them were highly abundant throughout the BSF treatment (30 days). These taxa could, in turn, consume the residual oxygen and create more anaerobic conditions, further suppressing pathogens (Wen et al. 2015). The present study showed that most fungi (including three potential pathogens) were positively correlated with the Eh value, indicating they were suppressed by the anaerobic/reductive conditions. This is consistent with the observation that most fungi cannot grow under anaerobic conditions (Takaya 2002). Furthermore, the increased pH and NH_4^+-N from BSF might mitigate soil acidification in the Sanqi cropping system. Therefore, soil pre-conditioned with BSF presented improved global physicochemical properties and was of higher quality than the initial soil, which may synergistically contribute towards inactivating pathogens to different extents. However, biogas slurry flooding (BSF) of soils is not always practical, particularly in ~~field, for instance~~ in hilly mountain areas. Hence, irrigating soil with BS to 100% water holding capacity (or less amount), similar with previous researches (Cao et al. 2016; Wen et al. 2015), may be an alternative to suppress pathogens.

In contrast, compared with those in the BSF-treated soils or initial soil, no significant changes in either the bacterial or fungal communities were observed in the WF treatment, as revealed by the number of culturable microorganisms and community structure via PCoA analysis. Furthermore, there was no obvious suppression of potential pathogens, including the culturable *Fusarium*, or reduction of root rot ratio. This was not consistent with previous reports indicating that WF results in highly efficient pathogen control (Kelman & Cook 1977; Niem et al. 2013). This implies that WF did not necessarily lead to significant shifts in the microbiota or pathogen suppression, further emphasizing the key roles of anaerobic microorganisms and organic matter derived from BS.

BSF reshaped the soil microbial community to a relatively pathogen-suppressive state

Throughout the experiment, BSF reshaped the bacterial and fungal community to form a different community structure (BS-related groups clustered together, as shown by PCoA and hierarchical cluster analysis; Fig. 3), which was consistent with a previous study showing that BSF application shifts the microbial community (Cao et al. 2016).

Importantly, BSF treatment reduced the abundance of potential pathogens (*Fusarium, Cylindrocarpon, Alternaria,* and *Phoma*), which might be the key factor contributing to the decrease of Sanqi root rot ratio. *Fusarium*, one of the main pathogens, was significantly reduced to a similar number by both CH and CF treatment, according to our plate count assay (Fig. 2d). However, ITS sequencing showed that the relative abundance of *Fusarium* was reduced in the CH treatment but increased in the CF group (Fig. 4b). Indeed, differences exist between culturable and culture-independent sequencing methods. The former is mainly affected by number of culturable microorganisms being detected by a specific medium, the latter is primarily affected by DNA extract method, primers used to amplify the 16S or ITS genes, sequence data analysis method, and so on (Bonk et al. 2018).

One possible reason was that CF treatment induced more anaerobic/reductive conditions (lower Eh value) than those in CH, which suppressed more total fungi while *Fusarium* with higher tolerance being less affected (Ebihara & Uematsu 2014), leading to the increasing ratio of *Fusarium* to fungi in CF compared CH (Fig. 2d). Meanwhile, other fungi except *Fusarium* were more suppressed by CF, resulting in the higher relative abundance of *Fusarium* in CF than in CH or Soil (Fig. 2d). In this study, the root rot ratio was positively correlated with the culturable *Fusarium* and ratio of *Fusarium*/fungi, which supported that the observed reduction of *Fusarium*/fungi is beneficial for growth of Sanqi (Zhao et al. 2017). One thing to be note is that taxa classified by OTUs are generally accurate at genus level, and some non-pathogenic *Fusarium* spp. contributed to the results but did not contribute to the occurrence of disease. As only culturable fungi can be enumerated by the plate count method, further absolute quantification methods, such as qPCR could be applied to verify this. Additionally, this study displayed a slightly negative correlation between the root rot ratio and culturable bacteria number, which was consistent with a previous study showing that total culturable bacteria is important for pathogen suppression (Bonanomi et al. 2010).

Overall, although potential pathogens in the soils were not completely eliminated, they were suppressed by other fungi or bacteria to lower levels under BSF treatment, with the potential to form a relatively pathogen-suppressive state (Cook 2014). This was further supported by the fungal functional prediction, where less “Plant Pathogen” and pathogenicity potential existed in the BSF-treated soils.

Commented [LMG12]: Insert discussion of methods from rebuttal (qPCR .. counting the number culturable *Fusarium* using Komada's selective medium (Line 189-191) in current study. This method has also been used by other researchers (e.g. **Wen et al., Journal of Soils and Sediments (2015) 16, 215-225; Tao et al., Microbiome (2020) 8, 137**), among which Tao et al. (2020) displayed that the results of qPCR and plate counting were positively correlated with each other”

BSF shifted bacterial community to harbor more beneficial taxa

BSF treatment led to significant changes in the bacterial community compared to that in the initial soil: *Synergistetes*, *Firmicutes,* and *Bacteroidetes* were enriched, while *Actinobacteria* and *Proteobacteria* were depleted. Members of *Synergistetes* can participate in synergistic acetate oxidation and digest organic acids to produce substrates of hydrogen methanogens, which play an important role in anaerobic metabolism (Acs et al. 2015). Bacteroidetes have been identified as beneficial taxa and are abundant in the rhizosphere of wild plants, to a certain extent; conversely, members of *Actinobacteria* and *Proteobacteria* are frequently enriched in the soils of pathogen-infected and long-term nitrogen-fertilized soils, being considered as marker taxa of poor soil quality (Dai et al. 2018; Perez-Jaramillo et al. 2018; Wu et al. 2016). A similar study showed that taxa of *Proteobacteria* and *Actinobacteria* are highly enriched in Sanqi monoculture soil (Zhao et al. 2017). Furthermore, Wu et al. (2016) considered *Proteobacteria* to be the marker taxa in Sanqi root rot soil. The declining abundance of *Proteobacteria* and *Actinobacteria* may reflect a positive effect of BSF treatment and indicate elevated soil micro-conditions against plant disease.

Under BSF treatment, the most enriched genera (e.g., *unidentified_Rikenellaceae*, *unidentified_Synergistaceae,* and *Sedimentibacter*) were mostly facultative/anaerobic. A previous study showed that application of BS to soil results in a change from active microbes to slower-growing anaerobes (Chen et al. 2012), which was consistent with the ~~findings of the~~ present study. Highly abundant anaerobic taxa have also been observed in maize-Sanqi rotation systems (Zhao et al. 2017), representing improved soil quality against disease. Furthermore, archaeal microorganisms (*Methanocorpusculum* and *Methanosaeta*), although present at a relatively low abundance, were enhanced. These facultative/anaerobic taxa may compete with the pathogenic fungi, or further create an anaerobic environment not suitable for them.

Bacterial community was vital for defending against pathogens

Plant roots interact with many microorganisms, including bacteria, fungi, and oomycetes in soil ~~environment~~, where fungi and oomycetes can usually cause serious disease, but the bacterial community is often negatively correlated with eukaryotic microbes and is vital for plant survival through defense against harmful root-related eukaryotes (Duran et al. 2018; Luo et al. 2020). Similarly, the present study demonstrated the culturable bacteria were slightly increased but culturable fungi were

significantly suppressed under BSF at the 30th day, similar to an earlier study (Walsh et al. 2012). Moreover, Spearman's correlation analysis revealed the presence of more negative correlations between the most abundant bacteria and fungi, as well as significantly negative correlations between numerous bacteria and the potential fungal pathogens. This indicates a potentially competitive or antagonistic relationship between these two groups. Thus, combined with the negative correlation between the number of culturable bacteria and root rot ratio, we considered that the bacterial community may play a vital role in suppressing fungal pathogens and controlling disease. Additionally, this study was conducted in a microcosm, without considering the interactions between plant roots and microbiota in the field. Pathogen levels might re-increase following planting, possibly owing to stimulation by specific root exudates (Li et al. 2014; Liu et al. 2018). Considering that pathogen resurgence is a risk after planting, this issue may be mitigated by introducing antagonistic bacteria alongside BS (Yin et al. 2021).

Culturable microorganisms could be used as an indicator linked with soil pathogenicity

~~Present~~~~The present study showed that the r~~Root rot ratio was positively correlated with the alpha-diversity (Shannon index) of the bacterial and fungal community. Higher microbial diversity is vital for community stability and pathogen suppression (van Elsas et al. 2012). However, this association is debatable; microbial diversity, e.g., relative abundance, might not serve as a credible indicator for supporting plant health (Huang et al. 2019; Xiong et al. 2017). In some cases, a higher microbial diversity community can harbor a lower biomass (i.e. absolute number of microbes in a certain environment) (Chen et al. 2017), which is consistent with the present study as shown by initial soil vs. CH0d (or CF0d) (harboring more microorganisms from BS). In present study, the highly abundant fungus OUT_1 in CH0d and CF0d resulted in unevenness in the community and, as expected, led to a decrease in the alpha-diversity indices. Alternatively, some absolute quantification measures, such as counts of culturable microorganisms in this study or combining with qPCR (Tao et al. 2020), could partially reflect the real diversity of the community and serve as a credible indicator of disease suppression.

Conclusions

To summarize, we created a conceptual graph to describe the influence of BS application on soil properties and microbial community structure (Fig. 7). The anaerobic/reductive environments created by BSF treatment elevated the soil physicochemical properties (e.g., pH, NH_4^+-N and Eh) and had a greater influence on the bacterial community. Following BSF treatment, bacteria were enriched while fungi were suppressed. In addition, PCoA, hierarchical cluster, and NTI analyses indicated that the bacterial community was more tightly clustered in the phylogenetic assembly. Together, these were responsible for assembling a stable bacterial community which negatively interacted with the fungal community, possibly by antagonism or competition for nutrients and niches, thereby suppressing the population of fungal pathogens and alleviating root rot. Taken together, this study provides a valuable reference for BS application, contributing towards alleviating the root rot disease in Sanqi production. ~~Further studies should be carried out to clarify the viability of pathogenic fungi under anaerobic conditions and evaluate the effects on Sanqi growth in the field.~~

Commented [LMG13]: Adds little

Acknowledgements

We would like to thank Editage for language editing.

References

Abubaker J, Risberg K, and Pell M. 2012. Biogas residues as fertilisers - Effects on wheat growth and soil microbial activities. *Applied Energy* 99:126-134. 10.1016/j.apenergy.2012.04.050

Acs N, Bagi Z, Rakhely G, Minarovics J, Nagy K, and Kovacs KL. 2015. Bioaugmentation of biogas production by a hydrogen-producing bacterium. *Bioresource Technology* 186:286-293. 10.1016/j.biortech.2015.02.098

Blok WJ, Lamers JG, Termorshuizen AJ, and Bollen GJ. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopathology* 90:253–259. doi.org/10.1094/PHYTO.2000.90.3.253

Bonanomi G, Antignani V, Capodilupo M, and Scala F. 2010. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biology and Biochemistry* 42:136-144. 10.1016/j.soilbio.2009.10.012

703 Bonk F, Popp D, Harms H, and Centler F. 2018. PCR-based quantification of taxa-
704 specific abundances in microbial communities: Quantifying and avoiding
705 common pitfalls. *Journal of Microbiological Methods* 153:139-147.
706 10.1016/j.mimet.2018.09.015
707 Cao Y, Chang Z, Wang J, Ma Y, Yang H, and Fu G. 2014. Potential use of anaerobically
708 digested manure slurry to suppress *Phytophthora* root rot of chilli pepper.
709 *Scientia Horticulturae* 168:124-131. 10.1016/j.scienta.2013.11.004
710 Cao Y, Wang J, Wu H, Yan S, Guo D, Wang G, and Ma Y. 2016. Soil chemical and
711 microbial responses to biogas slurry amendment and its effect on Fusarium wilt
712 suppression. *Applied Soil Ecology* 107:116-123. 10.1016/j.apsoil.2016.05.010
713 Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK,
714 Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights
715 D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M,
716 Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T,
717 Zaneveld J, and Knight R. 2010. QIIME allows analysis of high-throughput
718 community sequencing data. *Nature Methods* 7:335-336. 10.1038/nmeth.f.303
719 Chen C, Song X, Wei W, Zhong H, Dai J, Lan Z, Li F, Yu X, Feng Q, Wang Z, Xie H,
720 Chen X, Zeng C, Wen B, Zeng L, Du H, Tang H, Xu C, Xia Y, Xia H, Yang H,
721 Wang J, Wang J, Madsen L, Brix S, Kristiansen K, Xu X, Li J, Wu R, and Jia
722 H. 2017. The microbiota continuum along the female reproductive tract and its
723 relation to uterine-related diseases. *Nature Communications* 8:875.
724 10.1038/s41467-017-00901-0
725 Chen R, Blagodatskaya E, Senbayram M, Blagodatsky S, Myachina O, Dittert K, and
726 Kuzyakov Y. 2012. Decomposition of biogas residues in soil and their effects
727 on microbial growth kinetics and enzyme activities. *Biomass and Bioenergy*
728 45:221-229. 10.1016/j.biombioe.2012.06.014
729 Cook RJ. 2014. Plant health management: Pathogen suppressive soils. *Encyclopedia*
730 *of Agriculture and Food Systems*, 441-455.
731 Cristina G, Camelin E, Tommasi T, Fino D, and Pugliese M. 2020. Anaerobic digestates
732 from sewage sludge used as fertilizer on a poor alkaline sandy soil and on a peat
733 substrate: Effects on tomato plants growth and on soil properties. *Journal of*
734 *Environment Management* 269:110767. 10.1016/j.jenvman.2020.110767
735 Dahunsi SO, Oranusi S, Efeovbokhan VE, Adesulu-Dahunsi AT, and Ogunwole JO.
736 2021. Crop performance and soil fertility improvement using organic fertilizer

produced from valorization of *Carica papaya* fruit peel. *Scientific Reports* 11:4696. 10.1038/s41598-021-84206-9

Dai Z, Su W, Chen H, Barberan A, Zhao H, Yu M, Yu L, Brookes PC, Schadt CW, Chang SX, and Xu J. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of *Actinobacteria* and *Proteobacteria* in agro-ecosystems across the globe. *Global Change Biology* 24:3452-3461. 10.1111/gcb.14163

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, and Hacquard S. 2018. Microbial interkingdom interactions in roots promote arabidopsis survival. *Cell* 175:973-983. 10.1016/j.cell.2018.10.020

Ebihara Y, and Uematsu S. 2014. Survival of strawberry-pathogenic fungi *Fusarium oxysporum* f. sp. *fragariae*, *Phytophthora cactorum* and *Verticillium dahliae* under anaerobic conditions. *Journal of General Plant Pathology* 80:50-58. 10.1007/s10327-013-0476-0

Edgar RC. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nature Methods* 10:996-998. 10.1038/nmeth.2604

Hewavitharana SS, and Mazzola M. 2016. Carbon source-dependent effects of anaerobic soil disinfestation on soil microbiome and suppression of *Rhizoctonia solani* AG-5 and *Pratylenchus penetrans*. *Phytopathology* 106:1015-1028. 10.1094/PHYTO-12-15-0329-R

Huang X, Liu L, Wen T, Zhu R, Zhang J, and Cai Z. 2015a. Illumina MiSeq investigations on the changes of microbial community in the *Fusarium oxysporum* f.sp. *cubense* infected soil during and after reductive soil disinfestation. *Microbiological Research* 181:33-42. 10.1016/j.micres.2015.08.004

Huang X, Wen T, Zhang J, Meng L, Zhu T, and Cai Z. 2015b. Toxic organic acids produced in biological soil disinfestation mainly caused the suppression of *Fusarium oxysporum* f. sp. *cubense*. *BioControl* 60:113-124. 10.1007/s10526-014-9623-6

Huang X, Zhou X, Zhang J, and Cai Z. 2019. Highly connected taxa located in the microbial network are prevalent in the rhizosphere soil of healthy plant. *Biology and Fertility of Soils* 55:299-312. 10.1007/s00374-019-01350-1

Insam H, Gómez-Brandón M, and Ascher J. 2015. Manure-based biogas fermentation residues - Friend or foe of soil fertility? *Soil Biology and Biochemistry* 84:1-14.

10.1016/j.soilbio.2015.02.006

Kelman A, and Cook RJ. 1977. Plant pathology in the People's Republic of China. *Annual Review of Phytopathology* 15:409-429. 10.1146/annurev.py.15.090177.002205

Komada H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Review of Plant Protection Research* 8:114-124.

Kougias PG, and Angelidaki I. 2018. Biogas and its opportunities - A review. *Frontiers of Environmental Science & Engineering* 12:14-25. 10.1007/s11783-018-1037-8

Li X, Ding C, Hua K, Zhang T, Zhang Y, Zhao L, Yang Y, Liu J, and Wang X. 2014. Soil sickness of peanuts is attributable to modifications in soil microbes induced by peanut root exudates rather than to direct allelopathy. *Soil Biology and Biochemistry* 78:149-159. 10.1016/j.soilbio.2014.07.019

Li Y, Dai S, Wang B, Jiang Y, Ma Y, Pan L, Wu K, Huang X, Zhang J, Cai Z, and Zhao J. 2020. Autotoxic ginsenoside disrupts soil fungal microbiomes by stimulating potentially pathogenic microbes. *Applied and Environmental Microbiology* 86:e00130-00120. 10.1128/AEM.00130-20

Li Y, Wang B, Chang Y, Yang Y, Yao C, Huang X, Zhang J, Cai Z, and Zhao J. 2019. Reductive soil disinfestation effectively alleviates the replant failure of Sanqi ginseng through allelochemical degradation and pathogen suppression. *Applied Microbiology and Biotechnology* 103:3581-3595. 10.1007/s00253-019-09676-4

Liu HJ, Yang M, and Zhu SS. 2019. Strategies to solve the problem of Soil sickness of *Panax notoginseng* (Family: Araliaceae). *Allelopathy Journal* 47:37-56. 10.26651/allelo.j/2019-47-1-1218

Liu L, Chen S, Zhao J, Zhou X, Wang B, Li Y, Zheng G, Zhang J, Cai Z, and Huang X. 2018. Watermelon planting is capable to restructure the soil microbiome that regulated by reductive soil disinfestation. *Applied Soil Ecology* 129:52-60. 10.1016/j.apsoil.2018.05.004

Louca S, Parfrey LW, and Doebeli M. 2016. Decoupling function and taxonomy in the global ocean microbiome. *Science* 353:1272-1277. 10.1126/science.aaf4507

Luo L-F, Yang L, Yan Z-X, Jiang B-B, Li S, Huang H-C, Liu Y-X, Zhu S-S, and Yang M. 2020. Ginsenosides in root exudates of *Panax notoginseng* drive the change

of soil microbiota through carbon source different utilization. *Plant and Soil* 455:139-153. 10.1007/s11104-020-04663-5

Luo L, Guo C, Wang L, Zhang J, Deng L, Luo K, Huang H, Liu Y, Mei X, Zhu S, and Yang M. 2019. Negative plant-soil feedback driven by re-assemblage of the rhizosphere microbiome with the growth of *Panax notoginseng*. *Frontiers in Microbiology* 10:1597. 10.3389/fmicb.2019.01597

Miao ZQ, Li SD, Liu XZ, Chen YJ, Li YH, Wang Y, Guo RJ, Xia ZY, and Zhang KQ. 2006. The causal microorganisms of *Panax notoginseng* root rot disease (in Chinese). *Scientia Agricultura Sinica* 39:1371-1378.

Momma N, Kobara Y, and Momma M. 2011. Fe^{2+} and Mn^{2+}, potential agents to induce suppression of *Fusarium oxysporum* for biological soil disinfestation. *Journal of General Plant Pathology* 77:331-335. 10.1007/s10327-011-0336-8

Momma N, Kobara Y, Uematsu S, Kita N, and Shinmura A. 2013. Development of biological soil disinfestations in Japan. *Applied Microbiology and Biotechnology* 97:3801-3809. 10.1007/s00253-013-4826-9

Momma N, Yamamoto K, Simandi P, and Shishido M. 2006. Role of organic acids in the mechanisms of biological soil disinfestation (BSD). *Journal of General Plant Pathology* 72:247-252. 10.1007/s10327-006-0274-z

Mowlick S, Inoue T, Takehara T, Kaku N, Ueki K, and Ueki A. 2013. Changes and recovery of soil bacterial communities influenced by biological soil disinfestation as compared with chloropicrin-treatment. *AMB Express* 3:46. 10.1186/2191-0855-3-46

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, and Kennedy PG. 2016. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* 20:241-248. 10.1016/j.funeco.2015.06.006

Niem J, Gundersen B, and Inglis DA. 2013. Effects of soil flooding on the survival of two potato pathogens, *Sclerotinia sclerotiorum* and *Verticillium dahliae*. *American Journal of Potato Research* 90:578-590. 10.1007/s12230-013-9332-1

Perez-Jaramillo JE, Carrion VJ, de Hollander M, and Raaijmakers JM. 2018. The wild side of plant microbiomes. *Microbiome* 6:143. 10.1186/s40168-018-0519-z

Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, and Huttenhower C. 2011. Metagenomic biomarker discovery and explanation. *Genome Biology*

12:R60. 10.1186/gb-2011-12-6-r60

Stegen JC, Lin X, Konopka AE, and Fredrickson JK. 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. *The ISME Journal* 6:1653-1664. 10.1038/ismej.2012.22

Strauss SL, and Kluepfel DA. 2015. Anaerobic soil disinfestation: A chemical-independent approach to pre-plant control of plant pathogens. *Journal of Integrative Agriculture* 14:2309-2318. 10.1016/s2095-3119(15)61118-2

Takahashi S, Tomita J, Nishioka K, Hisada T, and Nishijima M. 2014. Development of a prokaryotic universal primer for simultaneous analysis of Bacteria and Archaea using next-generation sequencing. *PloS One* 9:e105592. 10.1371/journal.pone.0105592

Takaya N. 2002. Dissimilatory nitrate reduction metabolisms and their control in fungi. *Journal of Bioscience and Bioengineering* 94:506-510. 10.1016/s1389-1723(02)80187-6

Tang B, Dong Y, Wu K, He M, Liu J, Yin F, Zhang W, and Gong M. 2020. Crop rotation alleviates replant failure in *Panax notoginseng* (Burkill) F.H. Chen by changing the composition but not the structure of the microbial community. *Plant, Soil and Environment* 66:493-499. 10.17221/320/2020-pse

Tao C, Li R, Xiong W, Shen Z, Liu S, Wang B, Ruan Y, Geisen S, Shen Q, and Kowalchuk GA. 2020. Bio-organic fertilizers stimulate indigenous soil *Pseudomonas* populations to enhance plant disease suppression. *Microbiome* 8. 10.1186/s40168-020-00892-z

Toju H, Tanabe AS, Yamamoto S, and Sato H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *PloS One* 7:e40863. 10.1371/journal.pone.0040863

van Elsas JD, Chiurazzi M, Mallon CA, Elhottova D, Kristufek V, and Salles JF. 2012. Microbial diversity determines the invasion of soil by a bacterial pathogen. *Proceedings of the National Academy of Sciences of the United States of America* 109:1159-1164. 10.1073/pnas.1109326109

Walsh JJ, Rousk J, Edwards-Jones G, Jones DL, and Williams AP. 2012. Fungal and bacterial growth following the application of slurry and anaerobic digestate of livestock manure to temperate pasture soils. *Biology and Fertility of Soils* 48:889-897. 10.1007/s00374-012-0681-6

Wang B, Xia Q, Li Y, Zhao J, Yang S, Wei F, Huang X, Zhang J, and Cai Z. 2021. Root

rot-infected Sanqi ginseng rhizosphere harbors dynamically pathogenic microbiotas driven by the shift of phenolic acids. *Plant and Soil* 3. 10.1007/s11104-021-05034-4

Wen T, Huang X, Zhang J, and Cai Z. 2015. Effects of biological soil disinfestation and water regime on suppressing *Artemisia selengensis* root rot pathogens. *Journal of Soils and Sediments* 16:215-225. 10.1007/s11368-015-1172-9

Wu LJ, Zhao YH, Guan YM, and Peng SF. 2008. A review on studies of the reason and control methods of succession cropping obstacle of *Panax ginseng* C.A. Mey (in Chinese). *Special Wild Economic Animal and Plant Research* 2:68-72.

Wu Z, Hao Z, Sun Y, Guo L, Huang L, Zeng Y, Wang Y, Yang L, and Chen B. 2016. Comparison on the structure and function of the rhizosphere microbial community between healthy and root-rot *Panax notoginseng*. *Applied Soil Ecology* 107:99-107. 10.1016/j.apsoil.2016.05.017

Xiong W, Li R, Ren Y, Liu C, Zhao Q, Wu H, Jousset A, and Shen Q. 2017. Distinct roles for soil fungal and bacterial communities associated with the suppression of vanilla *Fusarium* wilt disease. *Soil Biology and Biochemistry* 107:198-207. 10.1016/j.soilbio.2017.01.010

Yang M, Zhang X, Xu Y, Mei X, Jiang B, Liao J, Yin Z, Zheng J, Zhao Z, Fan L, He X, Zhu Y, and Zhu S. 2015. Autotoxic ginsenosides in the rhizosphere contribute to the replant failure of *Panax notoginseng*. *PloS One* 10:e0118555. 10.1371/journal.pone.0118555

Yin C, Casa Vargas JM, Schlatter DC, Hagerty CH, Hulbert SH, and Paulitz TC. 2021. Rhizosphere community selection reveals bacteria associated with reduced root disease. *Microbiome* 9:86. 10.1186/s40168-020-00997-5

Yu G, Smith DK, Zhu H, Guan Y, Lam TTY, and McInerny G. 2017. ggtree: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution* 8:28-36. 10.1111/2041-210x.12628

Zhao J, Li YL, Wang BY, Huang XQ, Yang L, Lan T, Zhang JB, and Cai ZC. 2017. Comparative soil microbial communities and activities in adjacent Sanqi ginseng monoculture and maize-Sanqi ginseng systems. *Applied Soil Ecology* 120:89-96. 10.1016/j.apsoil.2017.08.002

Zhao J, Westerholm M, Qiao W, Yin D, Bi S, Jiang M, and Dong R. 2018. Impact of temperature and substrate concentration on degradation rates of acetate,

propionate and hydrogen and their links to microbial community structure. *Bioresource Technology* 256:44-52. 10.1016/j.biortech.2018.01.150

Zhou X, Li C, Liu L, Zhao J, Zhang J, Cai Z, and Huang X. 2019. Control of *Fusarium* wilt of lisianthus by reassembling the microbial community in infested soil through reductive soil disinfestation. *Microbiological Research* 220:1-11. 10.1016/j.micres.2018.12.001