Chemical and Biological Technologies in Agriculture

RESEARCH

Open Access

An optimized biofumigant improves pepper yield without exerting detrimental effects on soil microbial diversity

Setu Bazie Tagele[1,3], Ryeong-Hui Kim[2], Minsoo Jeong[1], Da-Ryung Jung[1], Dokyung Lee[2] and Jae-Ho Shin[1,2,3*]

Abstract

Background: Biofumigation is a non-chemical sustainable approach that reshapes soil microbiota to overcome challenges in way of continuous cultivation. However, the type and quantity of substrate have a significant impact on microbiota shifts and the subsequent success of biofumigation. Moreover, studies on the effects of biofumigant concentration in combination with fumigation duration on soil microbiota dynamics are very rare.

Research methods: We performed microcosm experiments to investigate how a biofumigant (Korean canola cultivar, HanRa) at various concentrations (0.5%, 1%, 2–4% w/w: biofumigant/soil) and fumigation periods (2–4 weeks) affects the soil bacterial and fungal communities. Subsequently, pot experiments employing two Korean canola cultivars (HanRa and YongSan) at 1% (w/w) were carried out.

Results: Illumina MiSeq analysis revealed that 2–4% biofumigant, regardless of incubation period, had a significant negative impact on microbial diversity and network complexity. In contrast, 1% biofumigant transformed the bacterial, fungal, and inter-kingdom networks into a highly connected and complex network without affecting microbial diversity. *Bacillus, Clostridium*, and *Pseudomonas* were the most highly stimulated bacterial genera in the biofumigated soils, whereas the abundance of Acidobacteria members was greatly reduced. The 2–4% amendments had substantially and more differentially abundant *Fusarium* than the 1%. Soil nutrition (e.g., pH, nitrate, ammonium, and exchangeable potassium), fruit yield, and weed suppression were enhanced in subsequent pot experiments. Of the nine soil chemical properties, phosphate and exchangeable potassium were the main factors influencing the microbial community assembly.

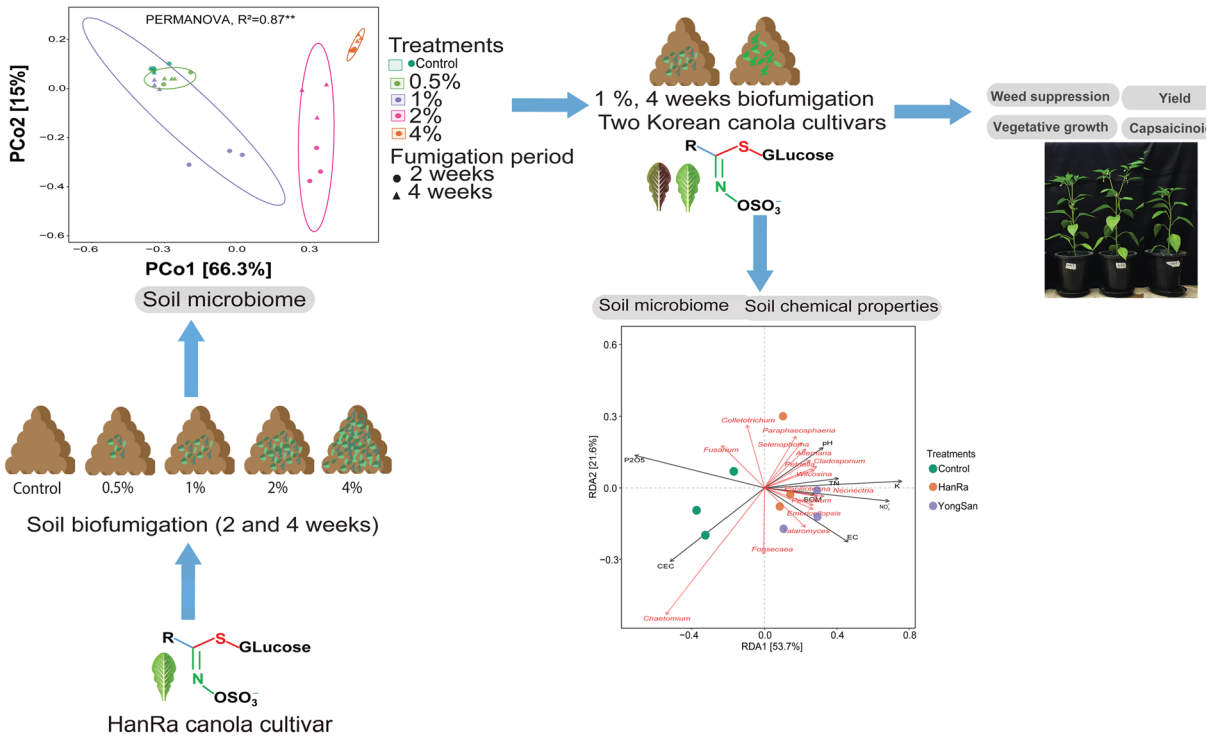
Conclusions: Optimized biofumigation-mediated increase in nitrate, ammonium, and potassium availability in the soil without causing any negative effects on soil microbial diversity indicates its potential as a preplant to improve crop productivity. This study contributes significantly to our understanding of how an optimal biofumigant can help ameliorate obstacles in continuous cropping.

Keywords: Biofumigant, Capsaicinoids, Illumina MiSeq, Pepper, Soil microbiota, Weed

*Correspondence: jhshin@knu.ac.kr

[1] Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea
Full list of author information is available at the end of the article

Graphical Abstract



Background

The long-term cultivation of high-value crops is a modern farming method to boost yield and meet ever-increasing consumer needs for food on limited land [1, 2]. Thus, high-value crops are cultivated intensively throughout the year with excessive use of fertilizers and pesticides [2]. However, these practices have resulted in nutrient imbalance, buildup of soil-borne pathogens, autotoxicity, soil acidification, heavy metal accumulation, and groundwater contamination [1]. These issues have raised concerns regarding the sustainability of continuous mono-cropping systems [3]. Chili pepper (*Capsicum annum*), a highly profitable crop farmed in many parts of the world, including South Korea, is plagued by obstacles in continuous pepper cultivation. To address soil nutrient imbalances, crop productivity declines, and biodiversity losses [4], the South Korean government has devised long-term soil management action plans [5]. Some solutions suggested to address the challenges of continuous farming include crop rotation, soil solarization, and organic amendments [6, 7].

Biofumigation entails creating an anaerobic soil environment with decomposable carbon biofumigants, irrigating to saturation, and covering it with plastic mulch for a 2–6 week period. Biofumigation is an environmentally friendly and sustainable alternative that improves soil fertility and crop productivity while reducing monocropping-related constraints [8]. Biofumigation is gaining popularity as a sustainable management option because it improves the biological and physicochemical properties of soil. Various volatile organic compounds such as acetic acid and butyric acid, which are toxic to soil-borne pathogens, weed seeds, and insect pests, are produced and accumulated as a result of organic matter decomposition [9, 10]. Biofumigation also creates a favorable environment for many beneficial microbes [10–12], which can potentially suppress the emergence of soil-borne bacterial and fungal pathogens and play an invaluable role in soil nutrient cycling and crop yield [11, 13]. However, the efficacy of biofumigation varies depending on the type of substrate and application rate [3, 9, 12]. For instance, Chen et al. [14] have reported that the inconsistent performance of biofumigation across different growing seasons was attributed to the quantity of the incorporated biomass. Furthermore, the impact of biofumigation duration on the taxonomic and functional diversity of soil microbial communities remains unclear. This suggests that additional research on the effects of biofumigant

concentration in combination with fumigation duration on soil microbiota dynamics is necessary.

Brassica species contain glucosinolates (GSLs), which are toxic to soil-borne pathogens, weeds, and insect pests. Thus, brassicas would exert better biofumigation effects, thereby boosting disinfestation efficiency and significantly alleviating the issues of continuous cultivation [12, 15]. In addition, although biofumigation improves soil health and productivity in many other crops [10, 13], little is known about its effects on pepper fruit pungency. Capsaicinoids, particularly capsaicin and dihydrocapsaicin, are the primary components that impart pungency to chili peppers [16]. Given that microbial communities are essential for plant health and plant productivity, we tested a hypothesis that the soil microbial community shift can be optimized using different concentration and fumigation periods. We also tested a hypothesis that an optimized biofumigation method may be applied to various biofumigants. Thus, we first carried out microcosm experiments to determine the impact of a biofumigant (Korean canola, HanRa) at various concentrations and fumigation periods on soil bacterial and fungal communities. The impact of optimized biofumigant concentration and fumigation duration (based on microcosm data) using two Korean canola cultivars (HanRa and YongSan), which had varying concentrations of GSLs, were then tested further in pot experiments on soil chemical properties, pepper productivity, fruit pungency, and soil microbiota.

Materials and methods

Experimental material

HanRa and YongSan canola cultivars were obtained from the National Institute of Crop Science (NICS), Rural Development Administration, South Korea. These canola cultivar seeds were sown, and biomass was harvested when the cultivars achieved approximately 50% blooming. The HanRa and YongSan canola cultivars had varying levels of total GSLs concentrations and nutrients, including nitrogen, phosphorus, and potassium (Additional file 1: Table S1). Field soil that had been subjected to pepper monocropping for many years and had low productivity was used for the microcosm and pot experiments. The soil was sourced from Gunwi-gun, Gyeongsangbuk-do Province, South Korea (36°10′09″N,128°38′24″E). The soil was sieved through an 8-mm sieve and homogenized.

Microcosm experiment

Microcosm experiments were performed to determine the effects of biofumigant concentration and fumigation duration on the soil bacterial and fungal community dynamics under controlled conditions. The soil was mixed with crushed biomass of the HanRa canola cultivar at rates of 0.5%, 1%, 2%–4% (w/w) on a dry weight basis in a small plastic container (70 mm (w) × 100 mm (l)). As a control, soil without biofumigant amendment was used. The mixes were watered at 70% water holding capacity with sterile distilled water and incubated independently for 2–4 weeks in a controlled environment (day/night cycle: 16/8 h, 22/18 °C, and 60% relative humidity). To inhibit volatilization of fumigants during biofumigation, the plastic containers were sealed. At the end of the experiment, the containers were opened, and the soil was air-dried for 60 d to dissipate the remnant toxic volatiles. Experiments had a completely randomized design with three replicates. One gram soil sample was taken from each treatment (different concentrations and fumigation periods) after 60 days of aeration and stored at − 80 °C until used for DNA extraction.

Pot experiment

Pot experiments were performed to determine the impact of optimized biofumigant concentration and fumigation duration (based on microcosm data) using two canola cultivars (HanRa and YongSan) on soil chemical properties, soil microbiota, and pepper plant growth performance. The same soil used for the microcosm experiments was mixed separately with the biomass of the HanRa and YongSan canola cultivars at a rate of 1% (w/w) on a dry weight basis. Non-amended soil served as a control. Triplicate plastic containers (50 cm (w) × 50 cm (l)) were filled with soil from each treatment and then watered to 70% field capacity with sterile distilled water. The soil was incubated for 30 days and air-drained, as mentioned in the microcosm experiments. After 60 days of aeration, 2 kg soil was placed into pots measuring 15 cm in diameter and 31 cm in height, with holes at the bottom. 1-month-old pepper seedlings of the cultivar Dongmudae were transplanted into each pot and grown for 3 months. Experiments had a completely randomized design with three replicates, each containing five pots (15 pots per treatment). The pepper plants were grown for 3 months after transplanting and were watered twice a week.

Soil samples for DNA extraction and chemical property analyses were collected immediately before pepper seedling transplantation. Soil sampling was performed from each pot by removing the top 2 cm soil. Soil samples from each pot were pooled and three random samples (replicates) were chosen for the analysis of soil microbiota and chemical properties. The samples for DNA extraction were stored at− 80 °C until use, and samples for chemical analysis were dried at room temperature.

Soil chemical property analysis

The soil chemical properties were analyzed at Kyungpook National University, South Korea, according to

Choe et al. [17]. The pH and electrical conductivity (EC) of soil samples were assessed using a pH and EC meter (SP2000, Skalar BV, Netherlands) from a soil suspension (1:5 (w/v)). Soil organic matter (SOM) content was determined using the titration method and an automatic titrator (Metrohm 888, Switzerland). Cadmium reduction [18] and salicylate [19] colorimetric methods were used to measure the concentrations of nitrate (NO_3^-) and ammonium (NH_4^+), respectively, on BLTEC QuAAtro (BLTEC KK, Osaka, Japan). The concentration of total nitrogen (TN) was measured using the method employed by Dumas [20] with S832DR (Leco, USA). The concentration of exchangeable potassium (K) in the soil was measured using a PerkinElmer Optima 8300 ICP-OES (PerkinElmer, Inc., MA, USA). The concentration of available P_2O_5 (AP) in the soil was measured using a SKALAR San + + system autoanalyzer (Skalar Analytical B.V., Breda, Netherlands). The $BaCl_2$–H_2SO_4 exchange method [21] was employed to measure the soil cation exchange capacity (CEC).

Weed suppression, pepper productivity, and fruit pungency

Pepper growth parameters, such as stem diameter, plant height, chlorophyll content, and primary branch length and diameter were measured. Chlorophyll concentration was determined using a chlorophyll meter (SPAD unit) (Konica Minolta, Japan). Weed germination, fruit yield, and fruit pungency were also assessed. Fully developed green pepper fruits on pots were harvested 3 times and fruit pungency was determined using freeze-dried pepper fruits. For this, a high performance liquid chromatography (HPLC) method as described by Han et al. [22] was used for the quantification of capsaicin and dihydrocapsaicin.

DNA extraction, library preparation, and sequencing

Microbial DNA from soil samples from the two experiments was extracted using the DNeasy PowerSoil® Pro Kit (Qiagen, Hilden, Germany) (0.5 g), according to the manufacturer's protocol. The purity of the extracted DNA was checked by gel electrophoresis and DNA was quantified using a Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The quality-checked DNA was stored at− 80 °C until used for sequencing.

The extracted DNA was used to amplify the V4–V5 hypervariable region of the bacterial 16S rRNA gene using the universal primers 515F/907R (5′-barcode-GTG CCAGCMGCCGCGGTAA-3′ and 5′-barcode-CCGYCA ATTCMTTTRAGTTT-3′) [23]. The fungal region targeting the ITS1 (internal transcribed spacer 1) was amplified using ITS86F/ITS4R primer pairs (5′-barcode-GTG AATCATCGAATCTTTGAA-3′ and 5′-barcode-TCC TCCGCTTATTGATATGC-3′) [24, 25]. The PCR conditions used are listed in Additional file 1: Table S2. The PCR mixture (50 µL) contained 25 µL EmeraldAmp® PCR Master Mix (Takara, Shiga, Japan), 1 µL DNA template, 1 µL (0.5 µM/µL) each primer, and 22 µL double-distilled water. The final PCR products were purified using AMPure XP beads (Beckman Coulter, CA, USA) and pooled at equimolar concentrations. Before loading the pooled library, the concentration and size of the library were checked on an Agilent Bioanalyzer (Santa Clara, CA, USA). Samples (final loading concentration: 7 pM) were sequenced on the Illumina MiSeq platform (Illumina) at Kyungpook National University's NGS Core Facility (Daegu, South Korea).

Bioinformatics analysis

Demultiplexing, denoising, chimera filtering, and truncating of bacterial and fungal raw sequences of each soil sample were performed using the QIIME2 pipeline (https://qiime2.org) and DADA2 [26]. After quality filtering non-biological sequences, representative sequences (amplicon sequence variants (ASVs)) were taxonomically assigned using a q2-feature-classifier trained on the reference SILVA 99% full-length database (version 138.1) [27] for bacteria and UNITE database (version 8.3) [28] for fungi. Taxonomic assignments of mitochondria, chloroplasts, and unclassified taxa at the kingdom level were excluded from downstream analysis. Sample reads were rarefied, and the rarefaction curve reached saturation (Additional file 1: Fig. S1), indicating that all samples had sufficient sequencing depth to estimate the diversity indices. Functional annotation of prokaryotic taxa (FAPROTAX) [29–31] and fungal functional guild (FUNGuild) [32] were used to predict the ecological functional changes of bacterial communities and fungal communities, respectively, at different biofumigant concentrations.

Statistical analysis

Statistical analyses of one-way and two-way ANOVA and data visualization were performed using R software (version 4.1.3) [33]. Levene's test and PERMDISP [34] [35] were used to check the homogeneity of variance and multivariate homogeneity of dispersion, respectively. The data normality assumption was tested using the Shapiro–Wilk test. ANOVA with the least significant difference (LSD) test was used to compare statistically significant differences among biofumigant concentration, and fumigation period of all alpha diversity indices, soil chemical properties, and pepper growth parameters using the dplyr package in R. Permutational multivariate analysis of variance (PERMANOVA) (Adonis; vegan, version 2.5.7) was employed to analyze the overall statistical variation in microbial community structure in response

to different treatments [36]. The relationship between soil microbiota and chemical properties was determined using distance-based redundancy analysis (dbRDA) in R. Differentially abundant bacteria that could serve as potential microbial biomarkers to distinguish biofumigated and non-amended control treatments were determined using LEfSe [37], metastat [38], metagenomeSeq [39], and random forest [40] in R. The random matrix theory (RMT) method was used to explore the co-occurrence network of microbial communities in different treatments. The method was based on Spearman's rank correlation method from microbial community compositional data at ASV level (>0.01%), with the correlation coefficient threshold set to 0.8 at $p \leq 0.05$.

Results

Microcosm experiment

Microbiota dynamics at different biofumigant concentrations and fumigation periods

There was a significant ($p \leq 0.05$) interaction between concentration (control, 0.5%, 1%, 2%, and 4%), fumigation period (2–4 weeks) on alpha and β diversity (Fig. 1, Table 1, and Additional file 1: Table S3). The lowest bacterial and fungal diversities were found following treatments with 2–4% biofumigant amendments during either of the fumigation periods. However, at 1% biofumigant with 4 weeks of fumigation, the fungal and bacterial diversities were not negatively affected. Furthermore, the recovery in bacterial diversity (Fig. 1a, b) after biofumigation (especially at 4%) was more noticeable than that in fungal diversity (Fig. 1c, d).

The soil bacterial and fungal community structures from 4% amendment, regardless of the fumigation duration, were highly distinct from those at other concentrations and were clustered separately. In addition, the 1–2% biofumigant amendments, particularly at 2 weeks of fumigation, exhibited different bacterial and fungal community structures and grouped apart from the control. In contrast, at 0.5% amendment, regardless of the fumigation duration, the fungal community, but not the bacterial community, clustered together with the community in the control sample, indicating a similar community structure profile (Fig. 1e, f).

The bacterial and fungal taxonomic compositions at the phylum level were significantly influenced by biofumigant concentration, fumigation duration, and their interactions (Fig. 1g, h, Additional file 1: Table S4). However, some phyla, such as Chloroflexi, Acidobacteriota, Nitrospirota, and Deinococcota, were only influenced by fumigant concentration. Bacteroidota was one of the most dominant phyla in all treatments during the 2-week fumigation, particularly at 4%, but it declined and was replaced by Bacillota at 4 weeks. The relative abundances of Acidobacteriota, Armatimonadota, Nitrospirota, and Verrucomicrobiota drastically reduced regardless of the fumigation period as the biofumigant concentration increased, especially at 2–4%. However, 1% biofumigant had no significant effect on Verrucomicrobiota, Nitrospirota, or Armatimonadota abundances compared to control. In addition, the abundances of Chloroflexi and Desulfobacteria were drastically reduced at 4%, whereas those of Pseudomonadota, Actinomycetota, and Deinococcota were highly enriched in comparison to the other treatments during both fumigation periods (Fig. 1g). Ascomycota dominated the fungal population for both fumigation durations at all fumigant concentrations. On the other hand, Basidiomycota were specifically and temporarily favored following the addition of biofumigant at a rate of up to 2% after 2 weeks of fumigation (Fig. 1h, Additional file 1: Table S4).

Differential taxon abundance after biofumigation

To identify potential microbial biomarkers that were differentially abundant following treatments with different biofumigant concentration and fumigation period, we used a variety of differential abundance tools; a random forest model, LEfSe analysis, metagenomeSeq, and metastat were used (Fig. 2, Additional file 1). Over 400 bacterial and 79 fungal taxa were significantly and differentially abundant between the biofumigant concentrations. Members of the p_Acidobacteriota, such as *Acidibacter* and *Vicinamibacteraceae*, which were enriched in the control, were less abundant after biofumigation, especially at higher concentrations, according to all the differential abundance tools used. On the other hand, biofumigation, particularly at 2% and 4% concentrations, stimulated members of Pseudomonadota and Bacteriodota such as *Castellaniella, Pseudomonas*, *Fermentimonas*, *Luteimonas*, and *Lysobacter*. Some beneficial genera, such as *Bacillus* and *Clostridium*, were more abundant at 0.5% and 1% amendment, moreover others, such as *Nitrospira,* were not adversely affected (Fig. 2a, c, Additional file 1: Fig. S2). *Fusarium* and *Botryotrichum* were differentially more abundant in 4% amendment, whereas *Gamsia, Chaetomium*, and *Apiotrichum* abundances were significantly reduced by the same treatment. In contrast, 1% biofumigant amendment had less relative abundance of *Fusarium*, whereas the same treatment significantly enriched *Apiotrichum* (Fig. 2b, d, Additional file 1: Fig. S2).

Microbial network complexity and functional diversity changes with biofumigation

Microbial co-occurrence network analysis aids in understanding the complex relationships among microbial communities in soil ecosystems. Thus, we performed an

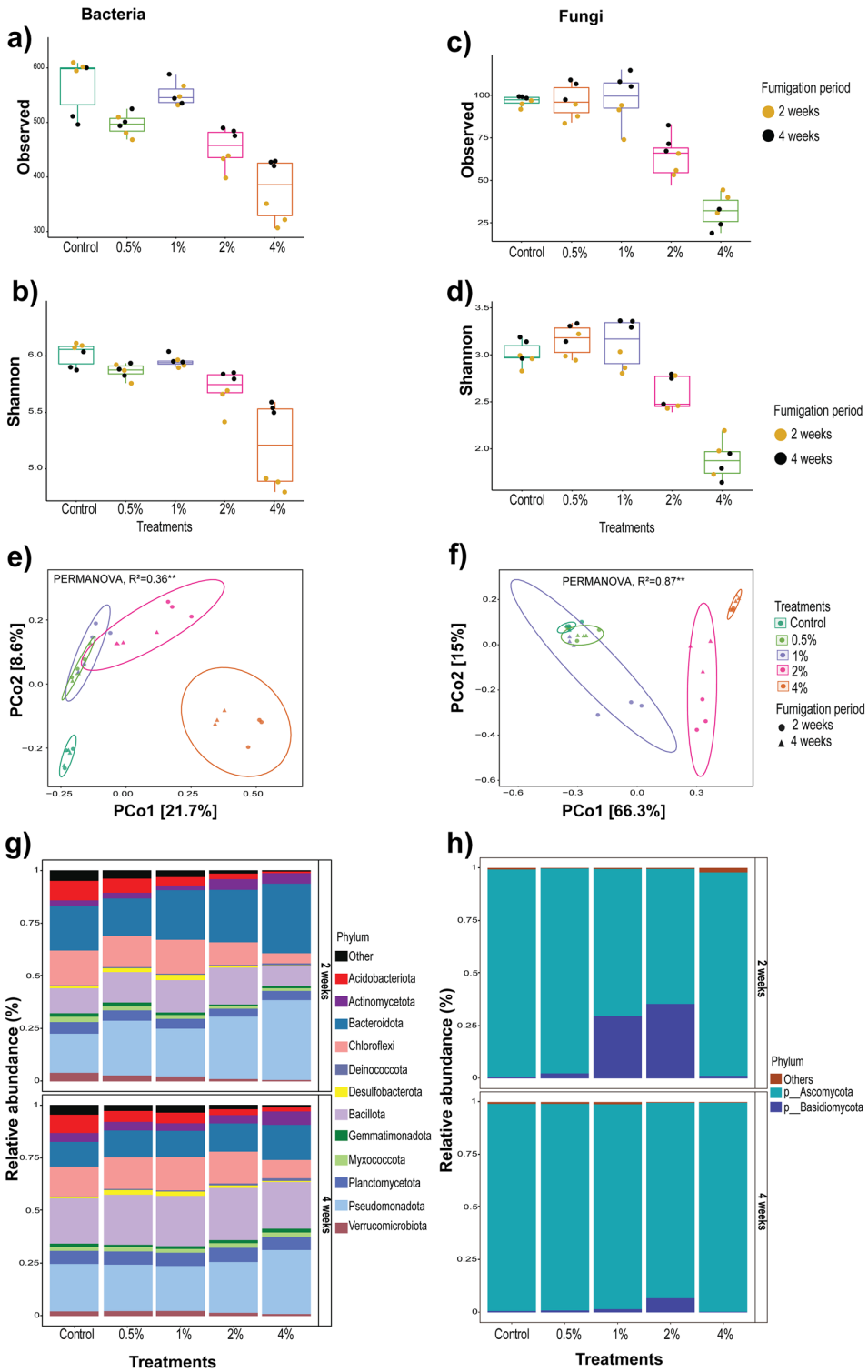


Fig. 1 Microbial diversity and community structure shift after biofumigation with different concentrations and fumigation periods. Observed and Shannon diversity indices of bacterial community (**a**, **b**) and fungal community (**c**, **d**) at 2 and 4 weeks of fumigation period with 0%, 0.5%, 1%, 2%, and 4% biofumigant concentrations. Principal coordinate analysis (PCoA) based on Bray–Curtis distance depicts the dissimilarity of bacterial (**e**, **f**) and fungal community structure at different concentrations of biofumigant amendment and fumigation periods. Phylum-level taxonomic composition of bacteria (**g**) and fungi (**h**) (> 0.1%)

Table 1 PERMANOVA analysis of the effects of biofumigant concentration and fumigation duration on bacterial and fungal community composition structure based on weighted uniFrac distance

Source of variation	df	16S F.Model	R^2	ITS F.Model	R^2
Concentration	4	3.91	0.36[c]	30.03	0.77[c]
Incubation period	1	2.49	0.06[b]	5.64	0.04[b]
Concentration [a] Incubation period	4	1.42	0.13[a]	2.67	0.07[a]
Residuals	20		0.45		0.128
Total	29		1.00		1.00

PERMANOVA: permutational multivariate analysis of variance. Incubation time: 2–4 weeks of biofumigant amendment to the soil. df: degree of freedom. 16S: bacterial community based on the V4–V5 hypervariable region of the 16S rRNA gene. ITS: fungal community based on the ITS1 region

[a] $p \leq 0.05$

[b] $p \leq 0.01$

[c] $p \leq 0.001$

RMT-based analysis to investigate how agricultural practices affect these relationships. Biofumigants at various concentrations caused remarkable variation in network topological properties and structure (bacteria–bacteria and fungi–fungi [intra-kingdom], and bacteria–fungi [inter-kingdom]) (Table 2 and Additional file 1: Tables S5, and S6, Fig. S3). Application of 1% biofumigant transformed the bacterial, fungal, and inter-kingdom networks into a highly connected and complex network, with a large number of nodes and links, and high average weighted degree (avWD), graph density (GD), and modules (Additional file 1: Fig. S3c). Conversely, 2–4% biofumigant reduced the intra- and inter-kingdom network complexities of the soil, characterized by a low number of nodes, links, avWD, and modules (Additional file 1: Fig. S3d and S3e).

FAPROTAX analysis was performed to evaluate the changes in the ecological functions of the bacterial communities following biofumigation. Forty-two predicted functions of the bacterial communities were noted in the biofumigated and non-amended controls. Among the predicted functions, chemoheterotrophy and aerobic chemoheterotrophy were the most abundant (Fig. 3a). More importantly, bacterial ecological functions of the soil subjected to 4% fumigation clustered separately from control and other treatments. Furthermore, LEfSe analysis shows that addition of 4% biofumigant was strongly associated with functions related to nitrogen cycling, chitinolysis, ureolysis, and animal_parasites_or_symbionts. Biofumigation enriched chemoheterotrophy, whereas the biofumigant non-amended control had more abundant functions related to phototrophy (Fig. 3b). We also used FUNGuild to predict changes in the ecological functions of the fungal communities after biofumigation (Fig. 3c). The saprotroph functional guild was the most dominant predicted function in all treatments, including the control group. The 1–2% treatments elevated soil ecological functions related to saprotrophs, especially during the first 2 weeks of fumigation. This mainly resulted from an increase in the abundance of Basidiomycota. At 2–4% biofumigant amendment, the predicted function of pathotroph was slightly diminished.

Pot experiment

Effect of optimized biofumigation on soil chemical properties and pepper productivity

The effects of two canola cultivars, HanRa and YongSan, at optimized concentrations and fumigation durations (1% for 4 weeks) on chemical properties, weed suppression, and plant growth are illustrated in Table 3. The addition of canola biofumigants significantly ($p \leq 0.05$) increased NO_3^-, NH_4^+, and K contents relative to the non-biofumigated control, even though the soil in all treatments was initially derived from a single composite soil sample. Compared to the non-amended control, HanRa and YongSan considerably improved the soil pH and EC contents, respectively. The control, which had a significantly ($p \leq 0.05$) lower pH than that of HanRa canola, showed high phosphate availability, which may be related to the high solubility of phosphate. However, the cation exchange capacity (CEC), TN, and SOM differences between canola biofumigated and non-biofumigated control soils were not statistically significant ($p > 0.05$). Based on these findings, we conclude that Korean canola cultivar amendments increased the overall nutritional status of the soil.

Furthermore, biofumigants significantly ($p \leq 0.05$) suppressed weed emergence and enhanced pepper yield (Table 4). Both monocot and dicot weed populations were significantly reduced after fumigation with the two canola cultivars. HanRa and YongSan canola cultivars increased pepper fruit yield by 49.8 and 55%, respectively, over control. More importantly, HanRa, followed by the control, showed the highest degree of pungency, as determined by the total concentrations of capsaicin and dihydrocapsaicin (Table 4).

Microbial diversity and composition changes following biofumigation with canola cultivars

The effects of the two canola cultivars on the bacterial and fungal alpha diversity indices are shown in Fig. 4a–d and Additional file 1: Table S7. Most diversity indices showed that the two canola cultivars had a strong positive effect on fungal diversity compared to the control. In addition, HanRa canola, as observed in the microcosm

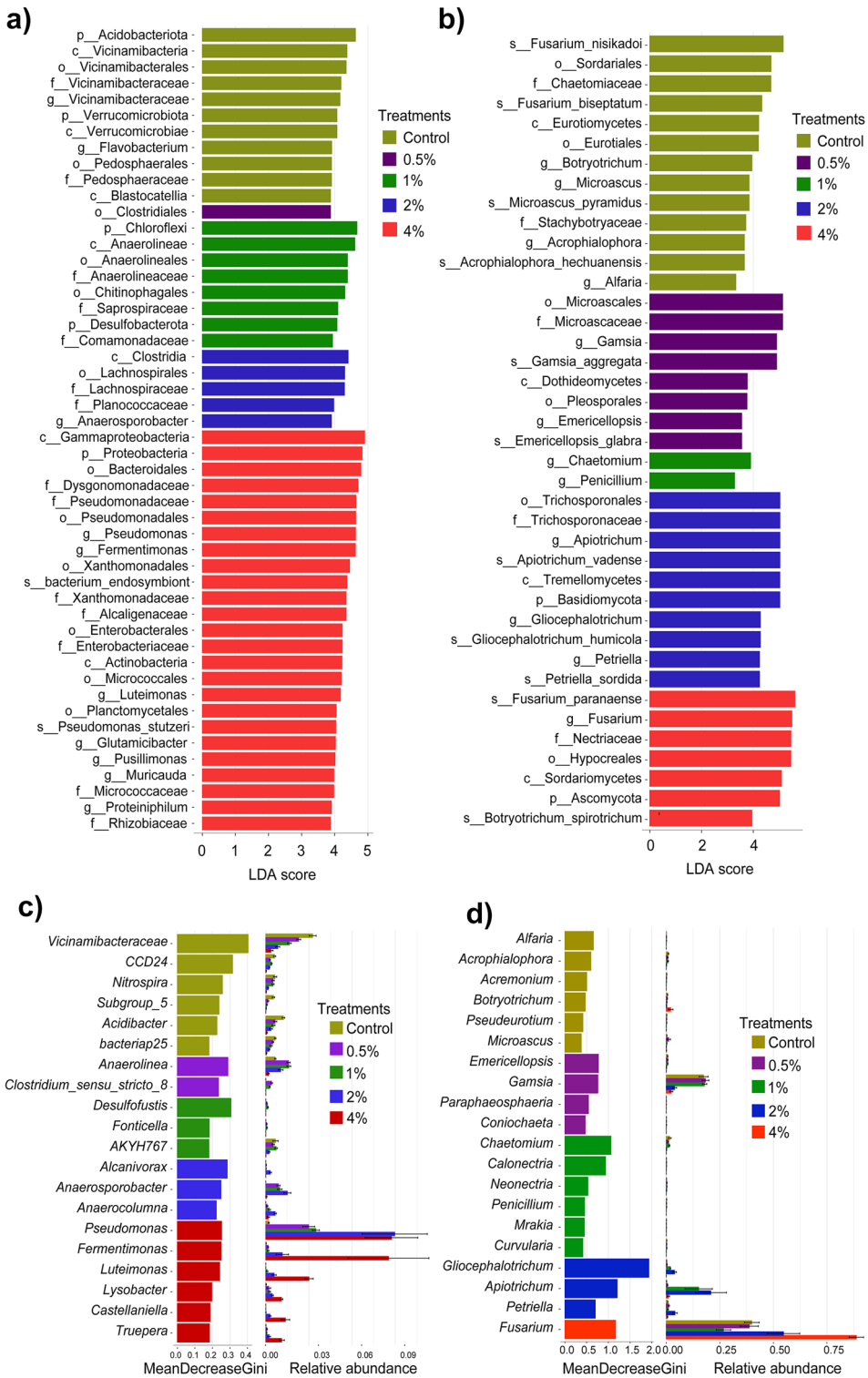


Fig. 2 Differentially abundant taxa following biofumigation at different concentrations and fumigation periods. Linear discriminatory analysis (LDA) and effect size (LEfSe) show the most significantly associated bacterial (**a**) and fungal (**b**) taxa with LDA score greater than 4 in different biofumigant concentrations and fumigation periods. Random forest analysis on the most predictive bacterial (**c**) and fungal (**d**) taxa as biomarkers for different biofumigant concentrations and fumigation periods. Taxon names are abbreviated as *p* phylum, *c* class, *o* order, *f* family, *g* genus, *s* species

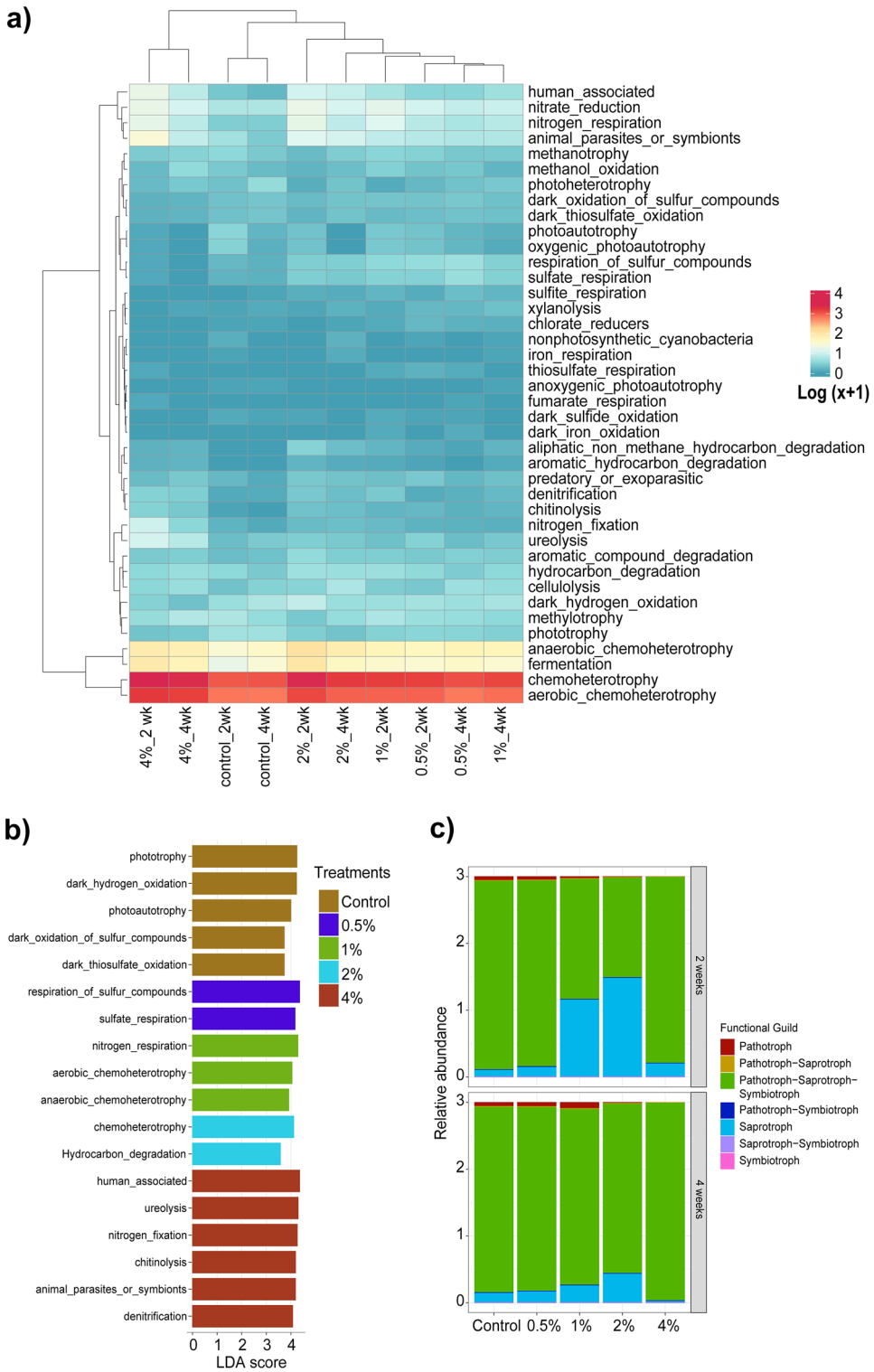


Fig. 3 Predicted ecological functions of bacterial and fungal communities. Ecological functions of bacterial communities at different concentrations and fumigation periods based on the FAPROTAX database (**a**). Predicted function fungal communities in different biofumigation treatments predicted using Funguild database (**b**)

Table 2 Co-occurrence network topological properties of bacteria–fungal communities at different biofumigation concentrations

	Biofumigation treatment				
	Control	0.5%	1%	2%	4%
Total nodes	839	972	1069	859	502
Total links[a]	8047	14763	13022	10913	3280
avWD[b]	19.182	30.377	24.363	25.408	13.068
Graph density (GD)	0.0229	0.0313	0.0228	0.0296	0.0261
Modularity	0.896	0.895	0.92	0.881	0.878
Modules	111	82	85	80	72

[a] Links: pairwise correlation of nodes

[b] Average weighted degree (avWD): average number of links per node

Table 3 Effects of Korean canola cultivars as biofumigant on soil chemical properties

Soil chemical properties	Treatments		
	Control	HanRa canola	YongSan canola
pH	6.17 ± 0.03^{b}	6.47 ± 0.03^{a}	6.23 ± 0.03^{b}
EC (dS m^{-1})	1.02 ± 0.00^{b}	0.48 ± 0.00^{c}	2.52 ± 0.01^{a}
CEC ($cmol_c$ kg^{-1})	17.50 ± 0.10^{a}	17.44 ± 0.40^{a}	16.88 ± 0.21^{a}
SOM (g kg^{-1})	26.23 ± 0.24^{a}	26.43 ± 0.40^{a}	26.26 ± 0.37^{a}
Total N (g kg^{-1})	1.6 ± 0.01^{a}	1.6 ± 0.00^{a}	1.7 ± 0.00^{a}
NO_3^- (mg kg^{-1})	6.40 ± 1.95^{c}	12.13 ± 0.55^{b}	18.30 ± 0.55^{a}
NH_4^+ (mg kg^{-1})	7.43 ± 0.23^{b}	9.37 ± 0.09^{a}	8.37 ± 0.20^{ab}
AP (mg kg^{-1})	687.30 ± 2.91^{a}	521.20 ± 1.91^{b}	534.95 ± 2.80^{b}
K ($cmol_c$ kg^{-1})	0.51 ± 0.01^{c}	1.00 ± 0.01^{b}	1.18 ± 0.01^{a}

Control: without biofumigant amendment. Mean values (n = 3) followed by different letter(s) in a row represent significant differences at $P \leq 0.05$, LSD test. Electrical conductivity (EC), cation exchange capacity (CEC), soil organic matter (SOM), total nitrogen (TN), nitrate nitrogen (NO_3^-), ammonium nitrogen (NH_4^+), available P_2O_5 (AP), exchangeable potassium (K)

experiments, had no negative impact on bacterial diversity (Fig. 4a, b, Additional file 1: Table S7). The two canola amendments also had different community structures for bacteria and fungi compared to the control (Fig. 4e, f).

The two canola cultivars had a remarkable impact on the taxonomic composition of the bacterial and fungal communities (Fig. 4g, h, Additional file 1: Table S8). The biofumigants had a significantly ($p \leq 0.05$) positive impact on the Bacillota population, but not on Acidobacteriota and Chloroflexi among the dominant phyla in the control (Fig. 4g, Additional file 1: Table S8). Clostridia dominated the soil bacterial communities in both canola biofumigants, whereas, in the control, they were rare members of Bacillota (Fig. 4g). In contrast, Acidobacteriae was negatively affected by both canola cultivars. The fungal community was dominated by Ascomycota, of which Chaetomiaceae was the most dominant family in all treatments (Fig. 4h). The effect of HanRa amendment of the Chaetomiaceae population was less detrimental than that of YongSan when compared to the control. In addition, the HanRa canola cultivar enriched fungal families, including Stachybotryaceae, Pyronemataceae, and Cladosporiaceae. HanRa amendment led to the highest abundance of Basidiomycota, with Rhynchogastremataceae being more enriched than in other treatments. Similar to the microcosm study, our pot experiments showed the positive effects of canola amendments on the relative abundance of *Bacillus*, whereas that of *Fusarium* was reduced (Additional file 1: Fig. S4a, b).

Relationships between soil microbial communities and chemical properties

Based on the Bray–Curtis distance, the Mantel test results illustrate the extent to which alterations in soil chemical properties during biofumigation affects the bacterial and fungal community structure assemblies (Table 5). The soil chemical properties explained 78.3–75.3% of total expected variation in bacterial and fungal community assemblies, respectively. The first component of RDA clearly separated the bacterial and fungal communities of canola cultivar-amended soil from those of the non-amended control (Fig. 5). Of the nine soil chemical properties examined, exchangeable K and available phosphate (AP) were significantly ($p \leq 0.05$) correlated with the community structure assemblies of both bacteria and fungi (Fig. 5, Table 5). Furthermore, the structure of the fungal community, but not of bacterial community, was significantly ($p \leq 0.05$) linked to soil NO_3^-, CEC, and EC (Table 5). The dbRDA analysis also shows that many genera of Bacillota, such as *Bacillus*, *Clostridium*, and *Fonticella*, were positively correlated with most soil nutrients, including NO_3^-, exchangeable K, and pH. In the fungal community, *Chaetomium* was one of the most influential genera following shift in soil chemical property after canola amendment. Mycorrhizal fungi, such as *Wilcoxina* and other Basidiomycota fungal genera, including *Papiliotrema*, were positively associated with most soil chemical properties, except CEC and AP. However, the relationship between *Fusarium* and soil chemical properties was in contrast to the findings discussed above.

Discussion

Soil microbial dynamics after biofumigation is concentration- and fumigation duration-dependent

Soil microbes are essential for nutrient cycling, soil fertility, crop protection, and productivity [41]. Biofumigation reshapes the soil microbiota [10, 13] via introduction or activation of beneficial microbes [12, 42, 43]. Our findings reveal that the 1% biofumigant at 4 weeks of fumigation had no negative impact on the bacterial and fungal

Table 4 Effects of Korean canola cultivars as biofumigant on weed emergence, pepper performance, and fruit pungency[a]

	Treatment		
	Control	HanRa canola	YongSan canola
Weed emergence (number/pot)			
Monocot	12.1 ± 0.5[a]	8.3 ± 0.9[b]	9.3 ± 0.3[b]
Dicot	11.9 ± 0.7[a]	7.3 ± 1.0[b]	7.7 ± 0.2[b]
Total	23.0 ± 0.7[a]	15.6 ± 1.5[b]	17.0 ± 0.4[b]
Plant height (cm)	527 ± 26.7[a]	573 ± 3.9[a]	547 ± 1.9[a]
Stem diameter (cm)	0.47 ± 0.02[a]	0.51 ± 0.01[a]	0.47 ± 0.02[a]
Chlorophyll contents (SPAD)	41 ± 0.8[b]	46 ± 1.4[a]	46 ± 0.8[a]
Canopy diameter (cm)	268 ± 23[a]	286 ± 5[a]	301 ± 8[a]
Primary branch length (cm)	5.9 ± 1.1[a]	7.0 ± 0.27[a]	7.4 ± 1.1[a]
Primary branch diameter (cm)	0.11 ± 0.02[a]	0.13 ± 0.01[a]	0.17 ± 0.02[a]
Fruit yield (g plant^{-1})	30.3 ± 4.0[b]	45.4 ± 3.2[a]	47.1 ± 3.8[a]
Pungency (mg 100 g^{-1})			
Capsaicin	178.0 ± 0.8[a]	171.7 ± 1.1[b]	109.5 ± 0.1[c]
Dihydrocapsaicin	77.7 ± 0.1[b]	91.5 ± 0.9[a]	50.6 ± 0.2[c]
Total pungency	255.7 ± 0.9[b]	263.2 ± 0.2[a]	160.1 ± 0.2[c]

[a] Weed emergence was collected a day before transplanting and pepper growth traits were determined at the end of the experiment. Fruit yield and pungency represented three fruit picking periods. Mean values followed by different letter(s) in a column represent significant differences at $p \leq 0.05$, LSD test

diversities, while improving the intra- and inter-kingdom network complexity of the soil. In contrast, the 2–4% biofumigant amendments, regardless of the fumigation period, reduced the microbial diversity and network complexity. Such varying responses of microbial diversity can be attributed partly to the direct toxicity of the hydrolysis products of canola amendments [44–46]. In addition, the incorporated biofumigants modify soil nutrients and microhabitats, thereby affecting soil microbial growth and colonization [47, 48] and contributing to the shift in soil bacterial and fungal community structure [13]. This is consistent with our results that exchangeable potassium and AP were the most important determining factors in shaping bacterial and fungal community structures. Such microbial communities that survive and flourish at 1% amendment could be highly resilient to environmental stresses [49], resistant to pathogen colonization [50], and maintain soil health [8] because of the more clustered and firmly connected microbial communities [51].

Biofumigant amendment creates favorable growth conditions for many members of copiotrophs, including *Bacillus* and *Clostridium* [9, 52, 53]. Similarly, our study showed that some members of Bacilliota were enriched after biofumigation. *Clostridium* is a diazotroph capable of nitrogen fixation [54] and is beneficial in suppressing soil-borne pathogens via releasing toxic organic acids [42]. On the other hand, a reduction in the relative abundance of Acidobacteria after soil amendments [9, 47] has been reported, which agrees with our findings. This may be partly attributed to the fact that the majority of Acidobacteriota are oligotrophic that adapt to the low availability of soil nutrients [55] and low pH [47]. Furthermore, the 1% biofumigant treatment, but not higher concentrations, was safe for these groups, including *Nitrospira*, indicating the need for biofumigation optimization. Members of Nitrospirota play key roles in regulating nitrogen uptake and improving plant growth [56, 57].

In the fungal community, the Basidiomycota population increased with the addition of biofumigants at a rate of up to 2%. Basidiomycota are cellulolytic fungi that play important roles in organic matter and litter decomposition. Thus, their enrichment after biofumigation is likely attributable to the incorporated biomass [58–60]. This is supported by the FUNGuild-predicted ecological function in which the saprotroph functional guild was temporarily elevated at 1–2% amendments. However, the decline in Basidiomycota abundance at 4% amendments may result from the high toxicity of the glucosinolate hydrolysis products during biofumigation [61, 62]. The relative abundance of *Fusarium,* an economically important pathogen with a wide host range that is common in long-term continuously pepper-cultivated soil [62, 63], was reduced with 1% amendment but increased in response to 2–4% biofumigant treatment. This is may be partly linked to decreased competition posed by biofumigant-sensitive soil microbes to the less sensitive *Fusarium* [64], as observed in response to 2–4% amendments that led to low bacterial and fungal alpha diversities. In addition, 2–4% biofumigants modified the soil microbiota by

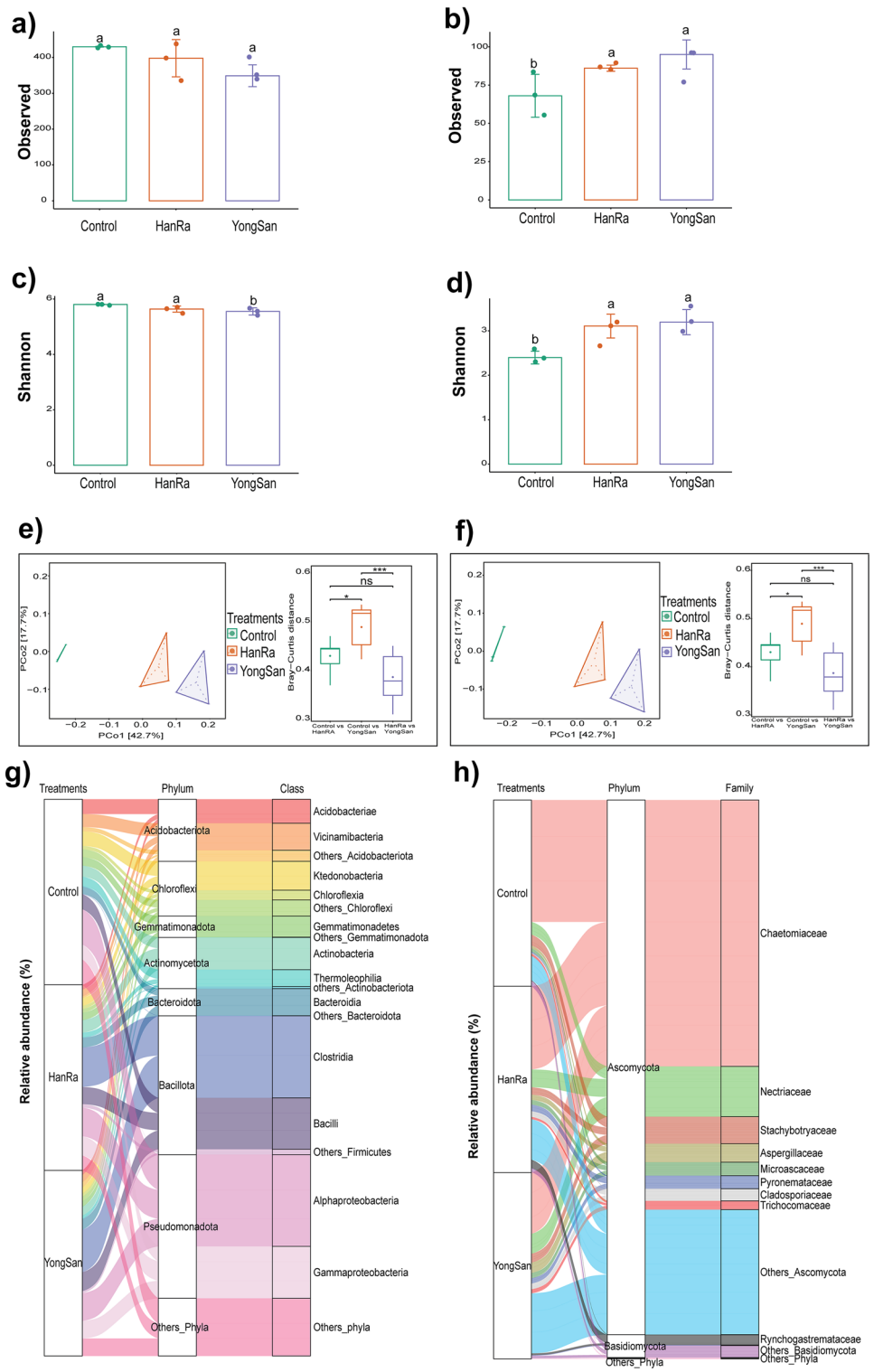


Fig. 4 Microbial diversity and community structure changes following biofumigation with two canola cultivars. Observed and Shannon diversity indices of bacterial community (**a**, **b**) and fungal community (**c**, **d**). Different letter(s) in each diversity index denotes statistically significant differences at $p \leq 0.05$ as determined using DMRT test. Principal coordinate analysis (PCoA) with Bray–Curtis distance showing the bacterial (**e**) and fungal (**f**) community structure shifts after biofumigation with two canola cultivars. Changes in bacterial (**g**) and fungal (**h**) taxonomic compositions (> 0.1%) after soil biofumigation with canola cultivars are shown

Table 5 Mantel test showing the correlation between microbial community structure (based on Bray–Curtis distance) and soil chemical properties

	Bacteria		Fungi	
	Correlation coefficient	Adjusted p value	Correlation coefficient	Adjusted p value
K	0.9108	0.009	0.7243	0.033
AP	0.8804	0.027	0.6352	0.033
NO_3^-	0.4657	0.059	0.4739	0.033
CEC	− 0.2094	0.898	0.3482	0.047
EC	0.0500	0.431	0.3522	0.047
SOM	− 0.2637	0.903	0.1250	0.226
TN	0.1048	0.431	− 0.0275	0.525
NH_4^+	0.4121	0.059	0.2929	0.089
pH	0.0568	0.431	− 0.0014	0.464

Exchangeable potassium (K), total nitrogen (TN), ammonium nitrogen (NH_4^+), available P_2O_5 (AP), soil organic matter (SOM), nitrate nitrogen (NO_3^-), electrical conductivity (EC), cation exchange capacity (CEC)

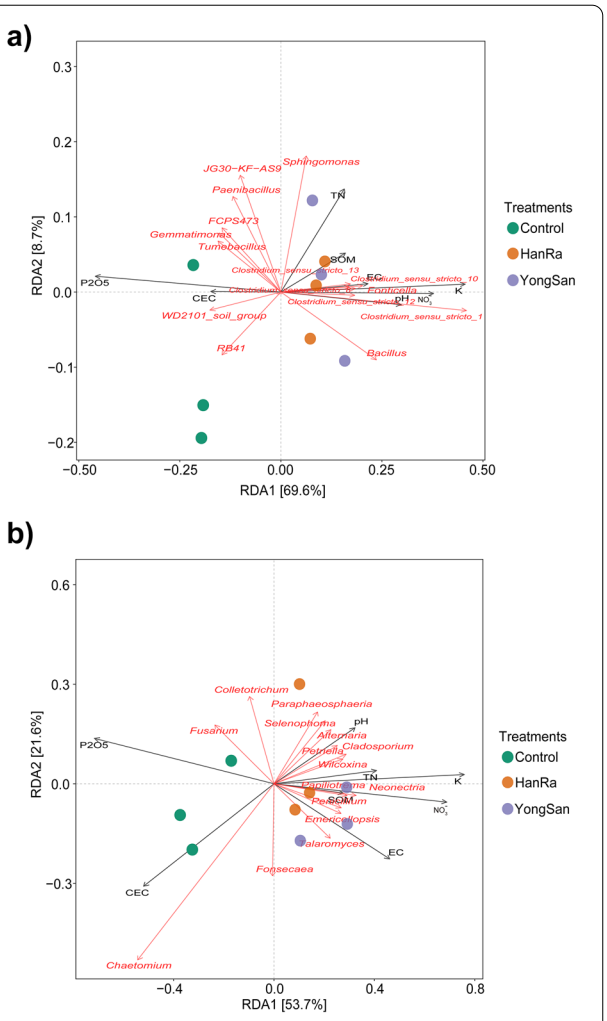


Fig. 5 Distance-based redundancy analysis (dbRDA, 1.5 scaling) exhibiting the relationship between soil chemical properties and microbial communities. Bacterial (**a**) and fungal (**b**) communities displaying the top 10 genera. Different colors represent biofumigants. The length of black arrows indicates the contribution of each soil chemical property to the variation in soil microbial community structure. The length of red arrows indicates the degree to which each soil chemical property influenced the microbial genera. The angles between different arrows represent correlations, where acute, obtuse, and right angles indicate positive, negative, and no correlations, respectively. Refer to Table 4 for the abbreviations of soil chemical properties

reducing the proportion of some beneficial soil microbes, such as *Bacillus*, which are often negatively correlated with *Fusarium* [65]. This suggests that microbiota-optimized biofumigation may aid in improving crop productivity via soil nutrient enrichment and suppression of soil-borne pathogens and weeds [41, 61, 66].

Biofumigation improves soil nutrition, weed suppression, and pepper performance

Biofumigation is a sustainable solution that improves crop productivity while reducing problems associated with mono-cropping. A significant increase in soil pH by HanRa canola amendment indicated its potential to ameliorate soil acidity by neutralizing the soil pH. A pH increase is associated with increased ammonification of biofumigated soils [67]. *Bacillus*, *Clostridium*, and *Pseudomonas*, whose abundance increased after biofumigation, may have contributed to ammonification [68]. Optimized biofumigation-mediated increase in nitrate, ammonium, and potassium availability in the soil without causing any negative effects on soil microbial diversity indicates its potential as a preplant to improve crop productivity. Similar reports have shown that biofumigants are rich sources of nutrients that enhance plant productivity and nourish soil microbes [69].

Our study results are also consistent with previous studies, which showed that biofumigation suppresses weeds [70], which could be attributed to microbe-mediated enhanced substrate decomposition that often results in the release of weed-suppressing organic acids [71], although further research is needed to confirm this hypothesis. Weeds are a major cause of increased cost in agriculture, necessitating a long-term and environmentally friendly weed control strategy [72]. In our study, biofumigation with canola cultivars had a positive effect on pepper yield. Several previous studies have also linked the high-yield performance of biofumigants to improved soil nutritional status [69, 73] and pathogen and weed suppression [70]. Pungency is an important sensory characteristic of hot peppers [16]. Capsaicin and dihydrocapsaicin are the two major pungency-imparting

chemicals that account for 69–22% of capsaicinoid content, respectively [16]. However, pungency varies with soil quality [74, 75]. Biofumigation with HanRa canola did not negatively affect the concentrations of capsaicin and dihydrocapsaicin, whereas that with YongSan led to the lowest concentrations of capsaicin and dihydrocapsaicin. The relationship between pungency and soil nutritional conditions is debatable, and further research is needed to clarify this issue [16, 74–76].

Conclusions

Our microcosm study results showed that using 1% biofumigant for 4 weeks had no negative impact on the bacterial and fungal diversities. In contrast, the 2–4% biofumigant amendments, regardless of the fumigation period, reduced the microbial diversity and network complexity. *Bacillus, Clostridium*, and *Pseudomonas* were the most highly stimulated genera in biofumigated soils, whereas the abundance of *Acidibacter* was reduced. In the fungal community, the 2–4% amendments, but not the 1%, significantly enriched relative abundance of *Fusarium*. Further pot experiments using two canola cultivars at optimized fumigation conditions (1–4 weeks) showed a positive effect on improving the soil nutritional status, suppressing weeds, and increasing pepper yield without negatively affecting soil microbial diversity. The major determinant factors in soil bacterial and fungal community structure assembly after biofumigation were exchangeable K and AP. This study contributes significantly to our understanding of how soil microbiota changes following treatment with different biofumigant concentrations and fumigation periods, and provides evidence that the optimized biofumigant can aid in overcoming obstacles for continuous cropping. Further research using other biofumigants at various concentrations of glucosinolate in diverse soil types is required to determine the efficiency of the optimized biofumigation.

Abbreviations

AP: Available phosphate; ASV: Amplicon sequence variant; avWD: Average weighed degree; CEC: Cation exchange capacity; EC: Electrical conductivity; GD: Graph density; GSLs: Glucosinolates; LSD: Least significant difference; RMT: Random matrix theory; SOM: Soil organic matter; TN: Total nitrogen.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-022-00365-5.

Additional file 1: **Table S1** Nutrient and total glucosinolate contents of biofumigants. **Table S2** Primers used in this study and PCR conditions used for Illumina sequencing. **Table S3.** Effects of biofumigant concentration and fumigation period on bacterial and fungal alpha diversity indices. **Table S4** Composition of bacterial and fungal communities at the phylum level following biofumigation at different concentrations and fumigation periods. **Table S5**. Co-occurrence network topological properties of bacterial communities at different biofumigant concentrations. **Table S6**. Co-occurrence network topological properties of fungal communities at different biofumigant concentrations. **Table S7**. Alpha diversity indices of bacterial and fungal diversity indices following soil fumigation with Korean canola cultivars. **Table S8** Composition of bacterial and fungal communities at the phylum level following Korean canola cultivar amendments. **Fig S1** Rarefaction curve of observed (**a**) bacteria and (**b**) fungi species in soil samples treated with different biofumigant concentrations and fumigation periods. **Fig S2.** Microbial community composition at the genus level in microcosm experiments. Bacterial (**a**) and fungal (**b**) community compositions following use of biofumigants at various concentrations and fumigation periods at the genus level. **Fig S3.** Co-occurrence networks of bacterial and fungal communities following canola biofumigation based on RMT analysis at ASVs level (Spearman's rank correlation (corr_cut = 0.7), $p < 0.05$): Control (**a**), 0.5% (**b**), 1% (**c**), 2% (**d**), and 4% (**e**). Node size in each treatment is proportional to the degree. **Fig S4.** Microbial community composition at the genus level in pot experiments. Bacterial (**a**) and fungal (**b**) community compositions at the genus level.

Acknowledgements

Not applicable.

Author contributions

SBT, R-HK and JHS planned and designed the research study; SBT, R-HK, D-RJ and D-KL performed the research; SBT, R-HK, M-SJ and J-HS analyzed the data; SBT, R-HK and D-RJ prepared figures and tables; SBT and J-HS wrote the manuscript. All authors read and approved the final manuscript.

Funding

This research was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea (NRF-2020R1I1A3074522 and NRF-2022R1I1A3071893) and the Korea Basic Science Institute (National Research Facilities and Equipment Center) financed by the Ministry of Education (NRF-2021R1A6C101A416), Republic of Korea. This research was also supported by the Ministry of Environment, Republic of Korea, for training professional personnel on biological materials.

Availability of data and materials

Under the PRJNA880449 BioProject, all raw sequences of bacteria and fungi are available at the NCBI Sequence Read Archive (SRA) repository (SRA accession SRR21577423- SRR21577473). The remaining data in this study are presented in tables and figures.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Applied Biosciences, Kyungpook National University, Daegu 41566, Republic of Korea. [2]Department of Integrative Biology, Kyungpook National University, Daegu 41566, Republic of Korea. [3]NGS Core Facility, Kyungpook National University, Daegu 41566, Republic of Korea.

Received: 15 September 2022 Accepted: 30 November 2022
Published online: 19 December 2022

References

1. Zhang H, Huang B, Dong L, Hu W, Akhtar MS, Qu M. Accumulation, sources and health risks of trace metals in elevated geochemical

background soils used for greenhouse vegetable production in southwestern China. Ecotoxicol Environ Saf. 2017;137:233–9.
2. Li J, Wan X, Liu X, Chen Y, Slaughter LC, Weindorf DC, et al. Changes in soil physical and chemical characteristics in intensively cultivated greenhouse vegetable fields in North China. Soil Tillage Res. 2019;195: 104366.
3. Chen D, Wang X, Carrión VJ, Yin S, Yue Z, Liao Y, et al. Acidic amelioration of soil amendments improves soil health by impacting rhizosphere microbial assemblies. Soil Biol Biochem. 2022. https://doi.org/10.1016/j.soilbio.2022.108599.
4. Chen W, Guo X, Guo Q, Tan X, Wang Z. Long-term chili monoculture alters environmental variables affecting the dominant microbial community in rhizosphere soil. Front Microbiol. 2021;12:1–15.
5. Jeon S, Jun H, Hyun B, Jung K, Sook M, Lee T, et al. Geoderma regional soil management priorities in Korea. Geoderma Reg. 2022;29: e00516.
6. Gao Y, Ren C, Liu Y, Zhu J, Li B, Mu W, et al. Pepper-maize intercropping affects the occurrence of anthracnose in hot pepper. Crop Prot. 2021;148: 105750.
7. del Guerrero MD, Lacasa CM, Martínez V, Martínez MC, Monserrat A, Larregla S. Low temperature biodisinfection effectiveness for *Phytophthora capsici* control of protected sweet pepper crops in the Southeast of Spain. Front Sustain Food Syst. 2021;5:1–8.
8. Liu S, Khan MH, Yuan Z, Hussain S, Cao H, Liu Y. Response of soil microbiome structure and its network profiles to four soil amendments in monocropping strawberry greenhouse. PLoS ONE. 2021;16:1–20.
9. Poret-Peterson AT, Albu S, McClean AE, Kluepfel DA. Shifts in soil bacterial communities as a function of carbon source used during anaerobic soil disinfestation. Front Environ Sci. 2019;6:1–15.
10. Lopes EA, Canedo EJ, Gomes VA, Vieira BS, Parreira DF, Neves WS. Anaerobic soil disinfestation for the management of soilborne pathogens: a review. Appl Soil Ecol. 2022. https://doi.org/10.1016/j.apsoil.2022.104408.
11. Huang X, Liu L, Wen T, Zhu R, Zhang J, Cai Z. Illumina MiSeq investigations on the changes of microbial community in the *Fusarium oxysporum f.sp*. cubense infected soil during and after reductive soil disinfestation. Microbiol Res. 2015;181:33–42.
12. Zhao J, Liu S, Zhou X, Xia Q, Liu X, Zhang S, et al. Reductive soil disinfestation incorporated with organic residue combination significantly improves soil microbial activity and functional diversity than sole residue incorporation. Appl Microbiol Biotechnol. 2020;104:7573–88.
13. Zhou X, Li C, Liu L, Zhao J, Zhang J, Cai Z, et al. Control of *Fusarium* wilt of *Lisianthus* by reassembling the microbial community in infested soil through reductive soil disinfestation. Microbiol Res. 2019;220:1–11.
14. Chen D, Zebarth BJ, Goyer C, Comeau LP, Nahar K, Dixon T. Effect of Biofumigation on population densities of *Pratylenchus spp.* and *Verticillium spp.* and potato yield in Eastern Canada. Am J Potato Res. 2022;99:229–42.
15. Mazzola M, Graham D, Wang L, Leisso R, Hewavitharana SS. Application sequence modulates microbiome composition, plant growth and apple replant disease control efficiency upon integration of anaerobic soil disinfestation and mustard seed meal amendment. J Crop Prot. 2020;132: 105125.
16. Bakpa EP, Xie J, Zhang J, Han K, Ma Y, Liu T. Influence of soil amendment of different concentrations of amino acid water soluble fertilizer on physiological characteristics, yield and quality of "Hangjiao No.2" chili pepper". PeerJ. 2021. https://doi.org/10.7717/peerj.12472.
17. Choe M, Hong SJ, Lim JH, Kwak Y, Back CG, Jung HY, et al. Korean paddy soil microbial community analysis method using denaturing gradient gel electrophoresis. J Appl Biol Chem. 2013;56:95–100.
18. Huffman SA, Barbarick KA. Soil nitrate analysis by cadmium reduction 1. Commun Sol Sci Plant Anal. 1981;12:79–89.
19. Kempers AJ, Zweers A. Ammonium determination in soil extracts by salicylate method. Commun Sol Sci Plant Anal. 1986;17:715–23.
20. Saint-Denis T, Goupy J. Optimization of a nitrogen analyzer based on the Dumas method. Anal Chim Acta. 2004;515:191–8.
21. Hendershot WH, Duquette M. A simple barium chloride method for determining cation exchange capacity and exchangeable cations. Soil Sci Soc Am J. 1986;50:605–8.
22. Han K, Jeong HJ, Sung J, Keum YS, Cho MC, Kim JH, et al. Biosynthesis of capsinoid is controlled by the Pun1 locus in pepper. Mol Breed. 2013;31:537–48.
23. Parada AE, Needham DM, Fuhrman JA. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol. 2016;18:1403–14.
24. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics PCR Protoc. Cambridge: Academic Press Inc; 1990.
25. Pryce TM, Palladino S, Kay ID, Coombs GW. Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. Med Mycol. 2003;41:369–81.
26. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13:581–3.
27. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013. https://doi.org/10.1093/nar/gks1219.
28. Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 2019;47:D259–64.
29. Louca S, Parfrey LW, Doebeli M. Decoupling function and taxonomy in the global ocean microbiome. Science. 2016;353:1272–7.
30. Sansupa C, Wahdan SFM, Hossen S, Disayathanoowat T, Wubet T, Purahong W. Can we use functional annotation of prokaryotic taxa (FAPROTAX) to assign the ecological functions of soil bacteria? Appl Sci. 2021;11:1–17.
31. Djemiel C, Maron PA, Terrat S, Dequiedt S, Cottin A, Ranjard L. Inferring microbiota functions from taxonomic genes: a review. Gigascience. 2022;11:1–30.
32. Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, et al. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 2016;20:241–8.
33. R Core Team. R: a language and environment for statistical computing. Vienna: R foundation for statistical computing; 2022.
34. Anderson MJ, Crist TO, Chase JM, Vellend M, Inouye BD, Freestone AL, et al. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecol Lett. 2011;14:19–28.
35. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHM, Szoecs E, Wagner H. Vegan: community ecology package: R package version 2.5-7. 2020. https://CRAN.R-project.org/package=vegan.
36. Dixon P. Computer program review VEGAN, a package of R functions for community ecology. J Veg Sci. 2003;14:927–30.
37. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.
38. White JR, Nagarajan N, Pop M. Statistical methods for detecting differentially abundant features in clinical metagenomic samples. PLoS Comput Biol. 2009. https://doi.org/10.1371/journal.pcbi.1000352.
39. Paulson JN, Colin Stine O, Bravo HC, Pop M. Differential abundance analysis for microbial marker-gene surveys. Nat Methods. 2013;10:1200–2.
40. Beck D, Foster JA. Machine learning techniques accurately classify microbial communities by bacterial vaginosis characteristics. PLoS ONE. 2014. https://doi.org/10.1371/journal.pone.0087830.
41. Ji L, Nasir F, Tian L, Chang J, Sun Y, Zhang J, et al. Outbreaks of root rot disease in different aged American ginseng plants are associated with field microbial dynamics. Front Microbiol. 2021;12:1–13.
42. Huang X, Wen T, Zhang J, Meng L, Zhu T, Cai Z. Toxic organic acids produced in biological soil disinfestation mainly caused the suppression of *Fusarium oxysporum f. sp. cubense*. Biocontrol. 2015;60:113–24.
43. Tagele SB, Kim RH, Shin JH. Interactions between *Brassica* biofumigants and soil microbiota: causes and impacts. J Agric Food Chem. 2021;69:11538–53.
44. Siebers M, Rohr T, Ventura M, Schütz V, Thies S, Kovacic F, et al. Disruption of microbial community composition and identification of plant growth promoting microorganisms after exposure of soil to rapeseed-derived glucosinolates. PLoS ONE. 2018;13:e0200160.

45. Zhu J, Ren Z, Huang B, Cao A, Wang Q, Yan D, et al. Effects of fumigation with allyl isothiocyanate on soil microbial diversity and community structure of tomato. J Agric Food Chem. 2020;68:1226–36.
46. Gao J, Pei H, Xie H. Influence of allyl isothiocyanate on the soil microbial community structure and composition during pepper cultivation. J Microbiol Biotechnol. 2021;31:978–89.
47. Ye G, Banerjee S, He JZ, Fan J, Wang Z, Wei X, et al. Manure application increases microbiome complexity in soil aggregate fractions: results of an 18-year field experiment. Agric Ecosyst Environ. 2021;307: 107249.
48. Shu X, He J, Zhou Z, Xia L, Hu Y, Zhang Y, et al. Organic amendments enhance soil microbial diversity, microbial functionality and crop yields: a meta-analysis. Sci Total Environ. 2022;829:154627.
49. He Q, Wang S, Hou W, Feng K, Li F, Hai W, et al. Temperature and microbial interactions drive the deterministic assembly processes in sediments of hot springs. Sci Total Environ. 2021;772: 145465.
50. Rybakova D, Mancinelli R, Wikström M, Birch-Jensen AS, Postma J, Ehlers RU, et al. The structure of the *Brassica napus* seed microbiome is cultivar-dependent and affects the interactions of symbionts and pathogens. Microbiome. 2017;5:104.
51. Shu X, He J, Zhou Z, Xia L, Hu Y, Zhang Y, et al. Organic amendments enhance soil microbial diversity, microbial functionality and crop yields: a meta-analysis. Sci Total Environ. 2022;829: 154627.
52. Liao H, Li Y, Yao H. Biochar amendment stimulates utilization of plant-derived carbon by soil bacteria in an intercropping system. Front Microbiol. 2019;10:1–13.
53. Li T, Liu T, Zheng C, Kang C, Yang Z, Yao X, et al. Changes in soil bacterial community structure as a result of incorporation of *Brassica* plants compared with continuous planting eggplant and chemical disinfection in greenhouses. PLoS ONE. 2017;12:e0173923.
54. Gao W, Wang L, Jia Z. Heterotrophy-coordinated diazotrophy is associated with significant changes of rare taxa in soil microbiome Pedosphere. Soil Sci Soc China. 2022;32:402–13.
55. Wang C, Liu D, Bai E. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol Biochem. 2018;120:126–33.
56. Li C, Hu HW, Chen QL, Chen D, He JZ. Comammox *Nitrospira* play an active role in nitrification of agricultural soils amended with nitrogen fertilizers. Soil Biol Biochem. 2019;138: 107609.
57. Zhu J, Li A, Zhang J, Sun C, Tang G, Chen L, et al. Effects of nitrogen application after abrupt drought-flood alternation on rice root nitrogen uptake and rhizosphere soil microbial diversity. Environ Exp Botany. 2022. https://doi.org/10.1016/j.envexpbot.2022.105007.
58. Wang F, Kong W, Ji M, Zhao K, Chen H, Yue L, et al. Grazing greatly reduces the temporal stability of soil cellulolytic fungal community in a Steppe on the Tibetan Plateau. J Environ Sci. 2022;121:48–57.
59. Li W, Lei X, Zhang R, Cao Q, Yang H, Zhang N. Shifts in rhizosphere microbial communities in *Oplopanax elatus* Nakai are related to soil chemical properties under different growth conditions. Sci Rep. 2022. https://doi.org/10.1038/s41598-022-15340-1.
60. Schmidt R, Mitchell J, Scow K. Cover cropping and no-till increase diversity and symbiotroph:saprotroph ratios of soil fungal communities. Soil Biol Biochem Elsevier. 2019;129:99–109.
61. Mazzola M, Hewavitharana SS, Strauss SL. *Brassica* seed meal soil amendments transform the rhizosphere microbiome and improve apple production through resistance to pathogen reinfestation. Phytopathology. 2015;105:460–9.
62. Ma Y, Gentry T, Hu P, Pierson E, Gu M, Yin S. Impact of brassicaceous seed meals on the composition of the soil fungal community and the incidence of *Fusarium* wilt on chili pepper. Appl Soil Ecol. 2015;90:41–8.
63. Liang Y, Gao Y, Wang R, Yang X. Fungal community characteristics and driving factors during the decaying process of *Salix psammophila* sand barriers in the desert. PLoS ONE. 2021;16:1–17.
64. Plaszkó T, Szűcs Z, Vasas G, Gonda S. Effects of glucosinolate-derived isothiocyanates on fungi: a comprehensive review on direct effects, mechanisms, structure-activity relationship data and possible agricultural applications. J Fungi. 2021. https://doi.org/10.3390/jof7070539.
65. Khan N, Maymon M, Hirsch AM. Combating *Fusarium* infection using bacillus-based antimicrobials. Microorganisms. 2017;5:1–13.
66. Wang T, Yang K, Ma Q, Jiang X, Zhou Y, Kong D, et al. Rhizosphere microbial community diversity and function analysis of cut *Chrysanthemum* during continuous monocropping. Front Microbiol. 2022;13:1–16.
67. Serrano-Pérez P, Rosskopf E, De Santiago A, del Rodríguez-MolinaC M. Anaerobic soil disinfestation reduces survival and infectivity of *Phytophthora nicotianae* chlamydospores in pepper. Sci Hortic. 2017;215:38–48.
68. Hui C, Wei R, Jiang H, Zhao Y, Xu L. Characterization of the ammonification, the relevant protease production and activity in a high-efficiency ammonifier *Bacillus amyloliquefaciens* DT. Int Biodeterior Biodegrad. 2019;142:11–7.
69. Di Gioia F, Ozores-Hampton M, Zhao X, Thomas J, Wilson P, Li Z, et al. Anaerobic soil disinfestation impact on soil nutrients dynamics and nitrous oxide emissions in fresh-market tomato. Agric Ecosyst Environ. 2017;240:194–205.
70. Shrestha U, Augé RM, Butler DM. A meta-analysis of the impact of anaerobic soil disinfestation on pest suppression and yield of horticultural crops. Front Plant Sci. 2016;7:1–20.
71. Tazawa J, Aoki D, Hayakawa H, Matsushima K-i, Nozoe T, Uchino A, Miura S, et al. Suppressive activity of volatile fatty acids and aromatic carboxylic acids on the germination of *Monochoria vaginalis*. Plant Prod Sci. 2021;24:505–11.
72. Hewavitharana SS, Mazzola M. Influence of rootstock genotype on efficacy of anaerobic soil disinfestation for control of apple nursery replant disease. Eur J Plant Pathol. 2020;157:39–57.
73. Di Gioia F, Ozores-Hampton M, Hong J, Kokalis-Burelle N, Albano J, Zhao X, et al. The effects of anaerobic soil disinfestation on weed and nematode control, fruit yield, and quality of Florida fresh-market tomato. HortScience. 2016;51:703–11.
74. Pascual I, Azcona I, Aguirreolea J, Morales F, Corpas FJ, Palma JM, et al. Growth, yield, and fruit quality of pepper plants amended with two sanitized sewage sludges. J Agric Food Chem. 2010;58:6951–9.
75. Das S, Sarkar S, Das M, Banik P, Bhattacharya SS. Influence of soil quality factors on capsaicin biosynthesis, pungency, yield, and produce quality of chili: an insight on Csy1, Pun1, and Pun12 signaling responses. Plant Physiol Biochem. 2021;166:427–36.
76. Antonious GF. Impact of soil management practices on yield, fruit quality, and antioxidant contents of pepper at four stages of fruit development. J Environ Sci Heal Part B. 2014;49:769–74.

Publisher's Note