Article

Not peer-reviewed version

Agro-Based Spent Mushroom Compost Substrates Improve Soil Properties and Microbial Diversity in Greenhouse Tomatoes

Chunguo Huang , Xiaoli Han , Qian Luo , Yuanjun Nie * , Min Kang , Yongjie Chen , Miaomiao Tang , Yayu Fu , Xiaoliang Li , Yinglong Chen

Posted Date: 18 July 2023

doi: 10.20944/preprints202307.1215.v1

Keywords: spent mushroom compost (SMC) substrate; cultivation years; physicochemical properties; enzyme activities; microbial community

Article

Agro-Based Spent Mushroom Compost Substrates Improve Soil Properties and Microbial Diversity in Greenhouse Tomatoes

Chunguo Huang [1], Xiaoli Han [2], Qian Luo [2], Yuanjun Nie [3,*], Min Kang [3], Yongjie Chen [4], Miaomiao Tang [1], Yayu Fu [1], Xiaoliang Li [1] and Yinglong Chen [5]

[1] College of Agriculture, Shanxi Agricultural University, Taigu 030801, China
[2] College of Forestry, Shanxi Agricultural University, Taigu, 030801 China
[3] College of Agricultural Economics and Management, Shanxi Agricultural University, Taiyuan 030031, China
[4] Institute of Wheat Research, Shanxi Academy of Agricultural Sciences/Shanxi Agricultural University, Linfen 041000, China
[5] The UWA Institute of Agriculture, and School of Agriculture and Environment, The University of Western Australia, Perth WA 6009, Australia
* Correspondence: yjnie@sxau.edu.cn; Tel.: +86-18636905379

Abstract: Spent mushroom compost (SMC) substrates are commonly used as growth media for greenhouse crops and horticulture production. This study aimed to investigate the responses of physiochemical soil properties, enzyme activities, and microbial community compositions to different cultivation durations and SMC soil treatments on tomatoes (*Solanum lycopersicum* L.) grown in plastic greenhouses on the Loess Plateau, China. The experiment included treatments: Two control treatments of non-planting SMC substrates (Sub CK) and continuous mono-cropping soil (Soil CK), and SMC substrate and the surrounding soil after planting at 1, 3, and 7 years. The results revealed that the SMC substrates had higher contents of major nutrients (e.g., total N and total P) than the surrounding soil treatments. The physicochemical soil properties and soil enzyme activities of the SMC substrates were significantly decreased with longer cultivation duration. Microbial alpha-diversity was higher in the SMC substrates regardless of cultivation duration than the control treatment (soil CK and substrate CK). Interestingly, following several years of tomato cultivation, the compositions of bacterial communities had more similarities with fungal communities in both the SMC substrates and surrounding soils. It was observed that many beneficial microbes, such as bacteria of the *Deinococcus-Thermus, Halanaerobiaeota,* and *Nitrospirae* phyla, and the fungi of the *Basidiomycota, Mortierellomycota,* and *Chytridiomycota* phyla were enriched in the SMC substrates. The pathogenic bacterial genus *Sphingomonas* and fungal genus *Fusarium* were abundant in the Soil CK treatment, while the potentially beneficial bacterial genera *Saccharimonadales, Gaiella, Bacillus*, and fungal genera *Thermomyces, Kernia*, and *Mortierella* were abundant in both the SMC substrate and surrounding soil. This study demonstrated that agro-based SMC substrates were a suitable growth media for a new grooved cultivation system.

Keywords: spent mushroom compost (SMC) substrate; cultivation years; physicochemical properties; enzyme activities; microbial community

1. Introduction

With rapidly intensifying climate change and an increasing global population, food security is a serious global scale issue [1]. The frequent occurrence of extreme weather events and imbalanced food supply patterns exacerbate the challenges that impact economic and social development worldwide [2,3]. To narrow the gap between food supplies and conventional open-field production, greenhouse technologies have been employed for the cultivation of crops and horticulture plants that can improve the production of off-season products [4]. Greenhouse cultivation has the capacity to finely control temperatures and microclimates to facilitate the year-round growth of horticulture plants to compensate for climatic instabilities in the field [5]. However, greenhouse technologies are a significant energy-intensive agricultural industry [6]. In the pursuit of high crop yields, surplus

 doi:10.20944/preprints202307.1215.v1

inputs of fossil-based mineral fertilizers can have deleterious effects on soil, leading to environmental contamination in greenhouse cultivation systems [7]. Hong et al. [8] suggested that excessive nutrient accumulation in plant root zones and downward leaching via irrigation result in soil and groundwater contamination in greenhouse cultivation systems. Furthermore, increased soil salinity, decreased crop yields, and continuous cropping obstacles (CCO) result in bottleneck issues for the development of the vegetable industry in greenhouses [9,10].

In seeking an alternative system to replace conventional greenhouse cultivation, researchers have invested more effort into potential alternatives. The application of agro-based spent mushroom compost substrates has been shown to be a beneficial alternative approach for the cultivation of crops and horticultural plants in greenhouse systems [11]. Spent mushroom compost (SMC) substrates are an effective cultivation media for vegetable production [12], which can reduce nitrogen loss in greenhouse systems [13]. The spent compost substrate consisted primarily of residual fungal mycelium, disintegrated lignocellulosic biomass, various nutrients, as well as organic matter and enzymes. Further, it had a low bulk density, loose texture, good air permeability, and nutrient retention. Thus, the SMC can be employed to bioremediate contaminated soil, and enhance its health [14] by improving the physical structure of the soil and ecological environment for soil microorganisms [15]. Furthermore, the SMC also contains a high organic matter content, phosphorus, and potassium, as well as trace elements that are required for plant assimilation and utilization [16]. Owing to its low level of toxic elements, strong absorption capacity, and enhanced soil aeration and water retention capacities, SMC has been utilized as a soil amendment that contributes to improving soil quality, agronomic efficiency, and environmental safety [15,17]. In addition, it contains several biologically active compounds such as therapeutically valuable polysaccharides and antibacterial peptides. These, together with its antibacterial properties protect plants against pathogens; thus, minimizing the incidence of plant diseases [18,19].

Under conventional greenhouse conditions, horticultural systems are subject to reduced soil organic matter, declining soil quality and fertility, and decreased microbial biomass [20–22]. Further, increased soil salinity, nutrient leaching, and soil compaction can result in continuous cropping obstacles (CCOs), which inhibit the prospects for increased production in greenhouse systems [8,10,23]. Although the use of SMC substrates as a biofertilizer, soil amendment, and for the bioremediation of pollution have attracted much attention [15], its role as a growth media for the cultivation of crops and horticultural plants in greenhouses is not well understood [11]. Unal [12] suggested that the application of SMC as a cultivation media exhibited positive impacts on the quality of tomato seedlings, plant growth, available organic matter, and nutrient uptake of plants [24,25]. Thus, there is a need to determine exactly how SMC influences the growth of tomato plants in greenhouses, inclusive of soil microbial communities and enzyme activities under different cultivation durations.

For this study, we investigated the impacts of SMC under varying cultivation timelines on physicochemical soil properties, soil enzyme activities, and the composition of microbial communities. We hypothesized that under greenhouse conditions: a) the application of agro-based SMC as growth media enhances the contents of major nutrients, enzyme activities, and microbial alpha-diversity in contrast to continuous mono-cropping soil; b) soil physicochemical properties and enzyme activities decrease with longer cultivation timelines; c) the composition of bacterial communities are more similar than fungal communities between the growth substrate and surrounding soil along with longer cultivation timelines; d) abundant beneficial microbes can be enriched by the SMC substrate and surrounding soil, which contribute to soil amelioration and improved plant growth.

2. Materials and Methods

2.1. Experimental design and soil sampling

This study was conducted in Hongtong County, Shanxi Province, on the Loess Plateau, in Northwestern China (35°87′84″N, 111°28′91″E). The annual temperature is 12.7℃, with 2079.1 hours

 doi:10.20944/preprints202307.1215.v1

of sunlight, 441.5 mm of precipitation, and 210 frost-free days. In May 2013, our group began to employ the SMC substrate as growth media to cultivate tomatoes (*Solanum lycopersicum* L.) in a plastic greenhouse. This compost consisted of spent mushroom compost cylinders of oyster mushroom (45% ~ 47%), cow manure (45% ~ 47%), and fragmented residues of tomato plants (6% ~ 10%). The SMC substrates were completely dried under sunlight and used as the growth media in a groove model system (Figures S1 and S2). Thus, various SMC substrate model cultivation durations were continually observed in the testing area. A non-planted substrate was also sampled prior to the experiment, which served as a control (Sub CK). Further, a continuous mono-cropping soil under greenhouse conditions was considered as another control treatment (Soil CK), to assess the effects of the SMC substrate amendment. We selected three different greenhouse tomato cultivation durations as the subjects of this study: Substrate after planting 1 year (Sub 1yr); surrounding soil of 1-yr substrate (Soil 1yr); substrate after planting 3 years (Sub 3yr); surrounding soil of 3-yr substrate (Soil 3 yr); substrate after planting 7 years (Sub 7yr); surrounding soil of 7-yr substrate (Soil 7 yr).

Each experimental plot was established in an independent greenhouse with an area of ~0.1 ha. All experimental plots received the same management based on the standard recommended practices for growing tomato plants in greenhouses. The physicochemical soil properties and soil microbe characteristics, as well as the surrounding soil (situated at the bottom and sides of the substrate) were quantified between all treatments. Five soil cores were extracted from each experimental plot (substrates and surrounding soil, respectively) using a soil-drilling sampler (10 cm inner diameter) on October 30, 2020. Five cores from the same plots were combined to form four mixed samples, for a total of sixteen soil samples and sixteen substrate samples. All samples were sifted through a 2 mm mesh sieve, after which each sample was divided in half. One half was stored in a 50 mL centrifuge tube with liquid N, and immediately transported to the laboratory pending DNA extraction. The other half was used for the quantification of physicochemical soil properties and soil enzyme activities.

2.2. Measurement of soil physicochemical properties and enzyme activities

The soil pH was determined from the supernatant of 0.01 M $CaCl_2$ soil slurries (1:1 (w/v)) after 10 min of vigorous shaking and soil particle settling [26]. The soil organic matter (SOM), total phosphorus (P), and total nitrogen (N) were determined via chromic acid titration, as well as the MADAC and Conway methods, respectively [27–29]. The soil organic carbon (C) was measured using an Isoprime isotope ratio mass spectrometer with a Eurovector elemental analyser [30]. Further, the soil alkaline phosphatase (ALP) enzyme activities were determined using a photometric technique [31]. The soil protease (Pro) and β-glucosidase (β-Glu) enzyme activities were calculated using a solid phase enzyme linked immunosorbent assay (ELISA) method with a protease ELISA Kit and β-Glucosidase ELISA Kit in soil infused biological fluids [32].

2.3. DNA extraction, PCR, and amplicon sequencing

The total microbial genomic DNA were extracted from 0.5 g of a soil sample using the OMEGA Soil DNA Kit (M5635-02) (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions [33]. Using a NanoDrop NC-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis, the quantity and quality, respectively, of extracted DNA were assessed. The PCR amplification of the bacterial 16S rRNA gene V3-V4 region was performed using the forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and the reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The fungal internal transcribed spacer (ITS) gene V1 region was determined using the forward primer (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and the reverse primer (5′-GCTGCGTTCTTCATCGATGC-3′). The PCR components contained 5 μL of buffer (5×), 0.25 μL of Fast pfu DNA polymerase (5 U/μL), 2 μL of 2.5 mM dNTPs, 1 μL (10 μM) of each forward and reverse primers, 1 μL of DNA template, and 14.75 μL of ddH_2O. The thermal cycling program proceeded as follows: initial denaturation at 98℃ for 5 min, followed by 25 cycles comprised of denaturation at 98℃ for 30 s, annealing at 55℃ for 30 s, and extension at 72℃ for 45 s, with a final extension of 5 min at 72℃. The PCR amplicons were purified using Vazyme VAHTSTM

 doi:10.20944/preprints202307.1215.v1

DNA Clean Beads (Vazyme, Nanjing, China) and quantified with a Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). The purified amplicons were pooled in equal amounts and paired-end sequenced (2×500 bp) using the Illumina MiSeq platform with MiSeq Reagent Kit v3 (Shanghai Personal Biotechnology Co., Ltd, Shanghai, China).

2.4. Data processing and bioinformatics analyses

Microbiome bioinformatics were run in QIIME2 2019.4 (http://docs.qiime2.org/2019.4/tutorials) according to the standardized protocols [34]. Briefly, raw FASTQ data were demultiplexed using the demux plugin followed by primer cutting with the cutadapt plugin [35], which were then quality filtered, denoised, and merged, with chimera removed using the DADA2 plugin [36]. The remaining high-quality amplicon sequence variants (ASVs) were submitted to the SRA (Sequence Read Archive) at the National Center for Biotechnology Information (NCBI) under accession number SUB12292980 (biosample SAMN31710080) for 16S bacteria sequences and number SUB12293105 (biosample SAMN31710019) for ITS fungi sequences. Non-singleton ASVs data were aligned with mafft [37] and used to construct a phylogeny with fasttree2 [38]. Alpha-diversity metrics (Chao1 [39], Shannon [40]), beta-diversity metrics (weighted UniFrac [41], unweighted UniFrac [42], and Bray-Curtis dissimilarity) were estimated using the diversity plugin. Taxonomy was assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier of the feature-classifier plugin [43] against the SILVA_132 database for bacteria, and UNITE_8.0 database for fungi [44].

Sequence data analyses were primarily performed using the QIIME2 and R packages (v3.2.0). The ASV-level alpha-diversity indices (Chao1 and Shannon) were calculated using the ASV table in QIIME2 and visualized as box plots. Beta-diversity analysis was performed to investigate the structural variations in microbial communities using Bray-Curtis metrics [45] and UniFrac distance metrics [41]. These were visualized using principal coordinate analysis (PCoA) and an unweighted pair-group method with arithmetic means (UPGMA) hierarchical clustering [46]. The significance of the differentiation of microbiota structures between groups was assessed by PERMANOVA (Permutational multivariate analysis of variance) [47], ANOSIM (Analysis of similarities) [48], and Permdisp [49] using QIIME2. The taxonomy compositions and abundances were visualized using MEGAN [50] and GraPhlAn [51]. A Venn diagram was created for visualization using the R package "VennDiagram" [52]. The relative abundances of taxa were statistically compared between samples or treatments by MetagenomeSeq, and visualized as Manhattan plots [53]. LEfSe (Linear discriminant analysis effect size) was performed to investigate differentially abundant taxa across groups using the default parameters [54]. Random forest analysis was performed to discriminate the samples from different groups using QIIME2 [55,56].

2.5. Statistical analysis

Data analysis was performed using SPSS statistics 19.0 software (SPSS Inc.). Analysis of variance was performed to assess the impacts of soil physicochemical properties, enzyme activities, microbial community diversity, and composition on the different treatments. The differences between means between the various treatments were calculated via the Tukey-HSD test at a probability level of 0.5 Spearman's rank correlations were used to correlate the physicochemical soil properties, enzyme activities, and soil microbial communities.

3. Results

3.1. Physicochemical soil properties

Significant influences of the cultivation duration were observed for the physicochemical properties of the SMC substrate and surrounding soil (Table 1). The organic matter, pH, and organic C content were significantly higher in the Sub 1yr treatment than the Sub 3yr and Sub 7yr treatments; however, there were no differences seen between Sub 1yr the Sub CK treatments. With increased cultivation duration, the organic matter, total N, and organic C content were significant decreased, with the lowest values being observed in the Sub 7yr treatment. The total P and pH were higher in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

the Sub 1yr treatment than the Sub 3- and 7yr treatments, which had no differences between them. For the surrounding soil, the Soil 3yr treatment possessed higher total P than the other treatments. The organic matter and organic C content were both highest under the Soil CK treatment and decreased with the cultivation duration, but showed no significant difference among them. Soil 1yr treatment exhibited the highest pH over the other treatments, which did not differ among themselves. Moreover, the total P, total N, organic matter, and organic C content were significantly higher for the substrate than in the surrounding soil with the cultivation duration (Table 1).

Table 1. Physicochemical soil properties of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the abbreviations of the treatments.

Types	Treatments	Total P (g/kg)	pH_{water}	Total N (g/kg)	Organic C (g/kg)	Organic matter (g/kg)
Substrates	Sub CK	2.85±0.06 c	7.81±0.07 a	19.24±0.65 a	38.87±7.83 a	67.02±13.50 a
	Sub 1yr	4.07±0.02 a	7.86±0.13 a	16.92±0.88 a	38.57±3.91 a	66.50±6.75 a
	Sub 3yr	3.11±0.04 bc	7.35±0.05 b	10.65±0.39 b	28.60±4.80 b	49.31±8.28 b
	Sub 7yr	3.22±0.04 b	7.27±0.05 b	6.11±0.38 c	16.70±1.43 c	28.83±2.46 c
Surrounding soil	Soil CK	1.34±0.04 e	7.45±0.06 b	1.81±0.02 d	33.97±0.46 ab	58.57±0.80 ab
	Soil 1yr	1.32±0.01 e	8.08±0.07 a	1.29±0.07 d	1.37±0.32 d	2.37±0.55 d
	Soil 3yr	1.74±0.08 d	7.54±0.05 b	2.27±0.09 d	4.35±0.70 d	7.50±1.21 d
	Soil 7yr	0.74±0.04 f	7.39±0.01 b	1.73±0.06 d	4.21±0.52 d	7.25±0.90 d

Data are means ± s.d. (n = 4), different letters means are significantly different at $p \leq 0.05$ (Tukey HSD).

3.2. Soil enzyme activities

The enzyme activities were significantly different between the substrates and surrounding soils (Table 2). Soil alkaline phosphatases (ALP), protease (Pro), and β-Glucosidase (β-Glu) enzyme activities were highest in the substrate for the Sub 1yr treatment and significantly decreased with the cultivation duration. For the surrounding soil, the soil ALP enzyme activities were higher in the Soil 3yr treatment and lower under the Soil 7yr treatment, whereas they did not differ for the soil CK. The Soil Pro enzyme activity was highest under the Soil 1yr treatment, but decreased significantly with longer cultivation durations. The soil β-Glu enzyme activity was highest in the Soil 7yr treatment than the other treatments. Further, the Soil Pro enzyme activities were significantly decreased with longer cultivation durations between the substrate and surrounding soil (Table 2). With longer cultivation durations, the soil β-Glu enzyme activities were significantly decreased in the substrates and conversely for the surrounding soil. The soil ALP enzyme activities had a higher peak value in the Sub 1yr and the Soil 3yr treatments.

Table 2. Enzyme activities of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the abbreviations of the treatments.

Types	Treatments	ALP (U/g)	Pro (IU/g)	β-Glu (U/g)
Substrates	Sub CK	0.64±0.01 cd	0.78±0.03 c	0.37±0.01 d
	Sub 1yr	0.85±0.02 a	1.61±0.07 a	0.51±0.01 a
	Sub 3yr	0.67±0.03 bcd	1.44±0.06 b	0.40±0.02 cd
	Sub 7yr	0.71±0.05 bc	0.77±0.05 c	0.28±0.02 e
Surrounding soil	Soil CK	0.61±0.03 d	0.42±0.02 d	0.30±0.02 e
	Soil 1yr	0.74±0.03 b	1.70±0.07 a	0.45±0.01 b
	Soil 3yr	0.92±0.02 a	0.83±0.08 c	0.44±0.01 bc
	Soil 7yr	0.61±0.03 d	0.49±0.03 d	0.50±0.01 a

Data are means ± s.d. (n = 4), different letters means are significantly different at $p \leq 0.05$ (Tukey HSD).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

3.3. Microbial alpha- and beta-diversity

The microbial alpha-diversity Chao1 and Shannon indices were significantly different between the substrate and surrounding soil under respective cultivation durations (Figure 1). In particular, the soil microbial (fungal and bacterial) α-diversity of the Shannon index were significantly different between the Sub CK and Sub 7yr treatments for the substrate (p = 0.021 and p = 0.0011, respectively). For the substrate, the bacterial α-diversity Chao1 and Shannon indices were significantly different between the Sub CK and Sub 3yr treatments (Figure 1a).

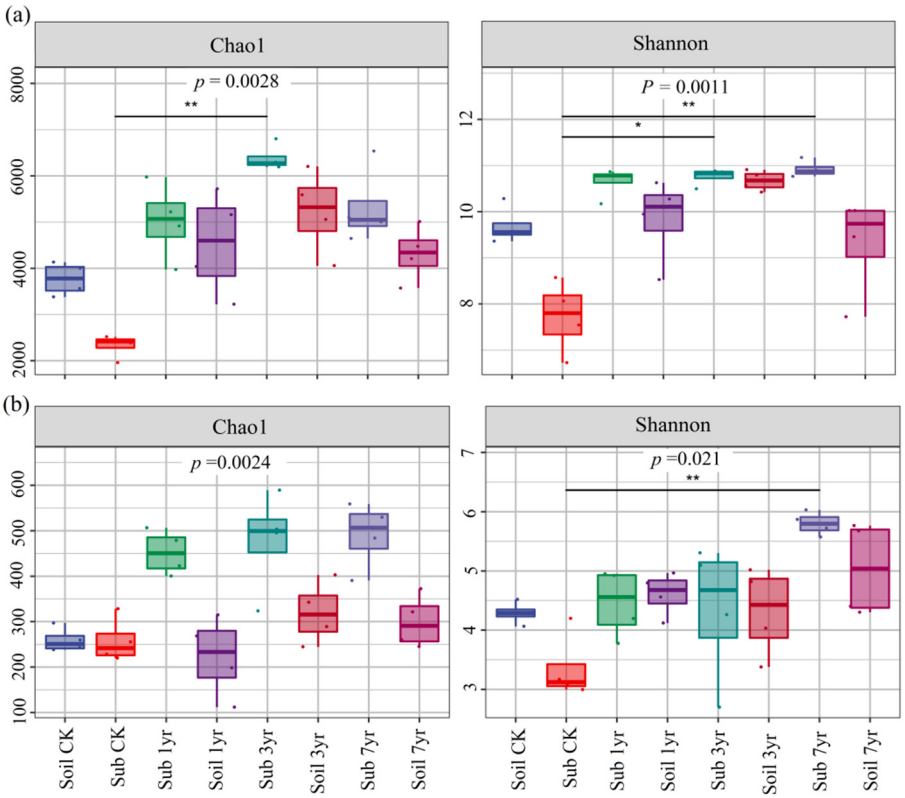

Figure 1. Bacterial (**a**) and fungal (**b**) taxa α-diversity indices of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the abbreviations of the treatments. (* Significant difference at $p \leq 0.05$ ** significant difference at $p \leq 0.01$).

The bacterial and fungal beta-diversity differed between the substrate and surrounding soil under the respective cultivation durations (Figure 2). The bacterial beta-diversity in the Sub CK and Soil CK treatments were significantly different from the other treatments. Following 1-, 3-, and 7-years of cultivation, the bacterial structural compositions of the substrates and in the surrounding soil exhibited a similar trend. The bacterial structural composition under the Sub 7yr treatment was quite similar to the other treatments (Figure 2a). The Sub 1-, 3-, and 7- year treatments differed in terms of their fungal community compositions in the substrates, and obviously differed from the Sub CK treatment (Figure 2b). Interestingly, the fungal structural compositions were not significantly different between Sub 7yr and Soil 3yr treatments.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

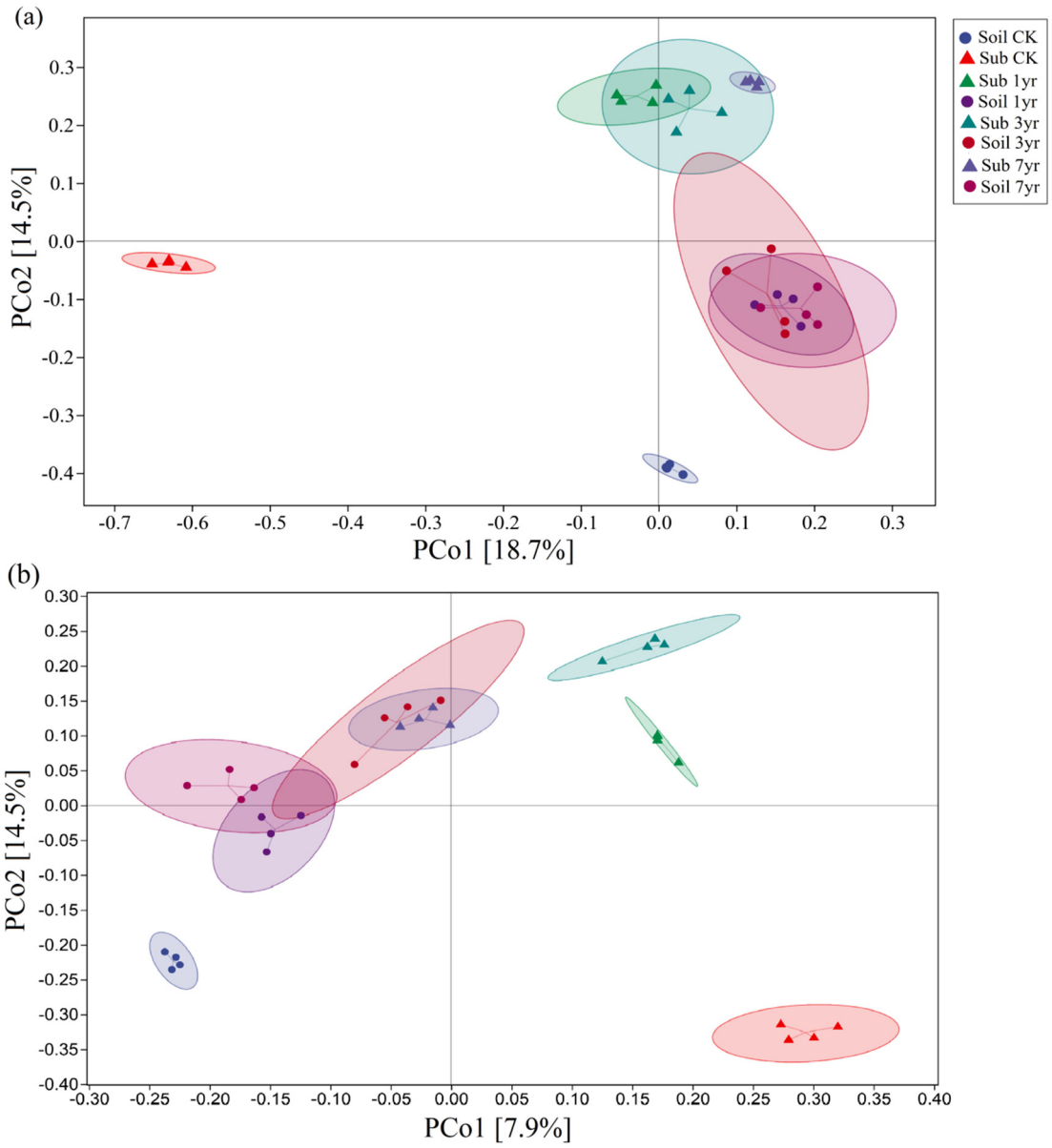

Figure 2. Bacterial (**a**) and fungal (**b**) Beta-diversity of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the abbreviations of the treatments.

3.4. Microbial community composition

For the substrates, the bacterial taxa of the top-10 most abundant phylum strongly fluctuated with the cultivation duration (Table 3, Figure S3). The Sub CK treatment possessed a higher abundance of *Chloroflexi*, *Bacteroidetes*, and *Deinococcus-Thermus* phyla than did the other treatments and had a decreased trend with longer cultivation durations. In contrast, the abundances of *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Gemmatimonadetes* phyla showed an increased trend with the cultivation duration, especially the *Acidobacteria* phyla. Further, the predominant fungal taxa of the top-5 most abundant phylum were significantly different between treatments. The abundance of the *Ascomycota* phylum was higher under the Sub 1yr treatment, while the *Mortierellomycota* phyla was higher under the Sub 3yr treatment. However, the Sub 7-year treatment exhibited a higher abundance of *Olpidiomycota* phylum than did the other treatments.

For the surrounding soil, the abundances of *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Cyanobacteria*, *Bacteroidetes*, and *Patescibacteria* bacterial phyla varied strongly between treatments (Table 3, Figure S3). The Soil 7yr treatment had a higher abundance of *Firmicutes*, the Soil 3-yr treatment showed higher abundances of *Chloroflexi* and *Patescibacteria* phyla, and the Soil CK treatment exhibited higher

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

abundances of *Cyanobacteria* and *Bacteroidetes* phyla. The dominant soil fungal taxa of *Ascomycota*, *Mortierellomycota*, and *Olpidiomycota* phyla were significantly different between treatments. The Soil 7yr treatment showed a higher abundance of *Mortierellomycota*, Soil 1yr treatment had a higher abundance of *Olpidiomycota*, and the Soil CK treatment had a higher abundance of *Ascomycota* phylum compared to the other treatments.

Table 3. Bacterial and fungal community composition of different cultivation duration of the substrate and the surrounding soil at the phyla level. See Table S1 for the abbreviations of the treatments.

Taxon	Phyla	Substrate				Soil			
		CK	1yr	3yr	7yr	CK	1yr	3yr	7yr
Bcteria	Acidobacteria	0.008 c	0.060 b	0.070 ab	0.069 ab	0.056 bc	0.090 a	0.090 a	0.042 bc
	Actinobacteria	0.166 c	0.261 ab	0.221 abc	0.264 a	0.187 abc	0.155 c	0.217 abc	0.172 bc
	Bacteroidetes	0.177 a	0.045 b	0.047 b	0.020 cd	0.046 b	0.018 d	0.026 c	0.017 d
	Chloroflexi	0.348 a	0.149 bc	0.132 cd	0.118 de	0.120 de	0.102 ef	0.193 ab	0.092 f
	Cyanobacteria	0.0002 e	0.001 c	0.0004 de	0.003 b	0.148 a	0.003 b	0.001 cd	0.004 b
	Deinococcus-Thermus	0.042 a	0.004 bc	0.004 bc	0.002 c	0.006 b	0.0002 d	0.004 bc	0.009 b
	Firmicutes	0.074 d	0.107 bcd	0.095 cd	0.159 bc	0.065 d	0.200 ab	0.059 d	0.392 a
	Gemmatimonadetes	0.010 c	0.025 b	0.030 b	0.028 b	0.070 a	0.038 b	0.063 a	0.032 b
	Patescibacteria	0.024 abc	0.034 ab	0.058 a	0.019 bc	0.013 cde	0.008 de	0.017 bcd	0.006 e
	Proteobacteria	0.140 c	0.300 a	0.319 a	0.295 ab	0.261 bc	0.357 a	0.296 ab	0.215 c
	Others	0.012 d	0.015 d	0.026 bc	0.024 bc	0.027 abc	0.029 ab	0.035 a	0.018 cd
Fungi	Ascomycota	0.250 c	0.727 a	0.242 c	0.465 abc	0.593 ab	0.349 bc	0.367 bc	0.327 bc
	Mortierellomycota	0.003 d	0.006 d	0.307 a	0.027 bc	0.012 cd	0.012 cd	0.0124 bc	0.206 ab
	Mucoromycota	0.0001 ab	0.0001 ab	0.001 a	0.0001 ab	0.000 b	0.000 b	0.0004 ab	0.0004 ab
	Olpidiomycota	0.000 c	0.002 ab	0.0001 bc	0.108 a	0.0003 c	0.138 a	0.0004 c	0.007 bc
	Rozellomycota	0.000 b	0.002 a	0.006 a	0.0002 b	0.000 b	0.0002 b	0.0001 b	0.0003 b
	Others	0.734 a	0.256 b	0.432 ab	0.387 b	0.386 b	0.499 ab	0.498 ab	0.450 ab

Data are means ± s.d. (n = 4), different letters means are significantly different at $p \leq 0.05$ in a line (Tukey HSD).

The heat map and clustering analysis of bacterial and fungal community compositions of the top-20 most abundant genera (Figure 3) revealed that the four dominant bacterial genera (*AKYG1722* (of *Chloroflexi* phyla), *A4b* (of *Chloroflexi* phyla), *Chryseolinea* (of *Bacteroidetes* phyla), and *SBR1031* (of *Chloroflexi* phyla)) (Figure 3a), and two fungal genera (*Melanocarpus* and *Sodiomyces* of the *Ascomycota* phyla) had significantly higher abundances under the Sub CK treatment (Figure 3b). The Sub 1yr treatment showed higher abundances of bacterial genera (*KD4-96* (*Chloroflexi* phyla), *Saccharimonadales* (*Patescibacteria* phyla), *67-14* (*Actinobacteria* phyla)), and fungal genera (*Melanocarpus*, *Ascobolus*, *Thermomyces*, and *Iodophanus* (*Ascomycota* phyla)). The Sub 3yr treatment had higher abundances of bacterial genera (*Saccharimonadales* (*Patescibacteria* phyla), *Nocardioides* (*Actinobacteria* phyla), *Subgroup_6* (*Acidobacteria* phyla), and *Gitt-GS-136* (*Chloroflexi* phyla)) (Figure 3a), and fungal genera (*Kernia* (*Ascomycota* phyla), *Mortierella*, and *Acaulium* (*Ascomycota* phyla)). The dominant bacterial genera (*67-14* (*Actinobacteria* phyla), *Nocardioides* (*Actinobacteria* phyla), *S085* (*Chloroflexi* phyla), and *Gaiella* (*Actinobacteria* phyla)), and fungal genera (*Remersonia*, *Aspergillus*, *Sodiomyces*, *Lophotrichus*, and *Fusarium* (*Ascomycota* phyla)) had significantly higher abundances under the Sub 7yr treatment (Figure 3b). Furthermore, the Soil CK treatment revealed higher abundances of the dominant bacterial genera (*Dongia* and *Sphingomonas* (*Proteobacteria* phyla)), and fungal genera (*Myceliophthora* and *Acremonium*) of the *Ascomycota* phyla (Figure 3). The Soil 1yr treatment had a higher abundance of bacterial *Sphingomonas* (*Proteobacteria* phyla), and two fungal genera (*Mycothermus* and *Pseudallescheria*) of the *Ascomycota* phyla. Two dominant bacterial genera (*JG30-KF-CM45* and *JG30-KF-CM66*) belonging to the *Chloroflexi* phyla, and fungal genera (*Remersonia* and *Kernia*) (*Ascomycota* phyla) were higher abundance under the Soil 3yr treatment. The Soil 7yr treatment had higher abundances of bacterial genera (*Paenisporosarcina* (*Firmicutes* phyla), *Bacillus* (*Firmicutes* phyla), *Gaiella* (*Actinobacteria* phyla), and *Dongia* (*Proteobacteria* phyla)) (Figure 3a), and fungal genera (*Aphanoascus*, *Stachybotrys*, *Acremonium*, *Pseudallescheria*, *Aspergillus*, and *Mortierella*) of the *Ascomycota* phyla (Figure 3b).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

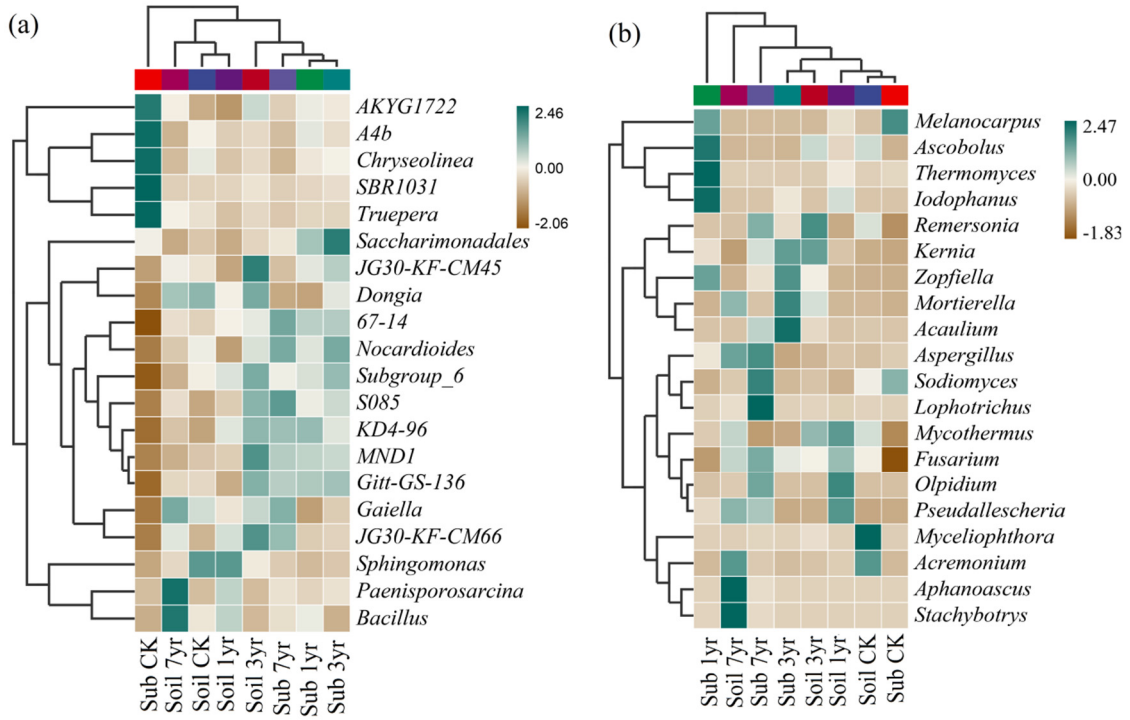

Figure 3. Heat map and clustering analysis of bacterial (**a**) and fungal (**b**) community composition of the top-20 most abundant genera of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the treatments.

3.5. Biomarker taxa analyses

Random Forests Based Importance Analysis of the top-10 most abundant phyla (Figure 4), revealed that bacterial *Deinococcus-Thermus* and *Bacteroidetes* phyla, and fungal *Basidiomycota* phyla were identified as the biomarker taxa of the Sub CK treatment with obviously higher relative abundances in the samples. Bacterial *Dependentiae* and *Patescibacteria* phyla, and fungal *Ascomycota* and *Rozellomycota* phyla were biomarker taxa of the Sub 1yr treatment. Bacterial *Patescibacteria, Dependentiae* and *Nitrospirae* phyla, and fungal *Mortierellomycota, Rozellomycota*, and *Mucoromycota* phyla were biomarker taxa of the Sub 3yr treatment. The biomarker taxa of the Sub 7yr treatment were the bacterial *Halanaerobiaeota* phyla and fungal *Chytridiomycota* and *Olpidiomycota* phyla. Moreover, for the surrounding soil, biomarker taxa of the bacterial *Verrucomicrobia* and *Armatimonadetes* phyla and fungal *Ascomycota* phylum were more highly abundant under the Soil CK treatment. Bacterial *Firmicutes, Dependentiae, Nitrospirae, Rokubacteria,* and *Armatimonadetes* phyla, and fungal *Chytridiomycota* and *Olpidiomycota* phyla were biomarker taxa of the Soil 1yr treatment. Bacterial *Rokubacteria, Nitrospirae*, and *Dependentiae* phyla, and fungal *Aphelidiomycota* and *Calcarisporiellomycota* phyla were biomarker taxa of the Soil 3yr treatment. Bacterial *Firmicutes* phyla and fungal *Blastocladiomycota* and *Mortierellomycota* phyla were biomarker taxa in the Soil 7yr treatment.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

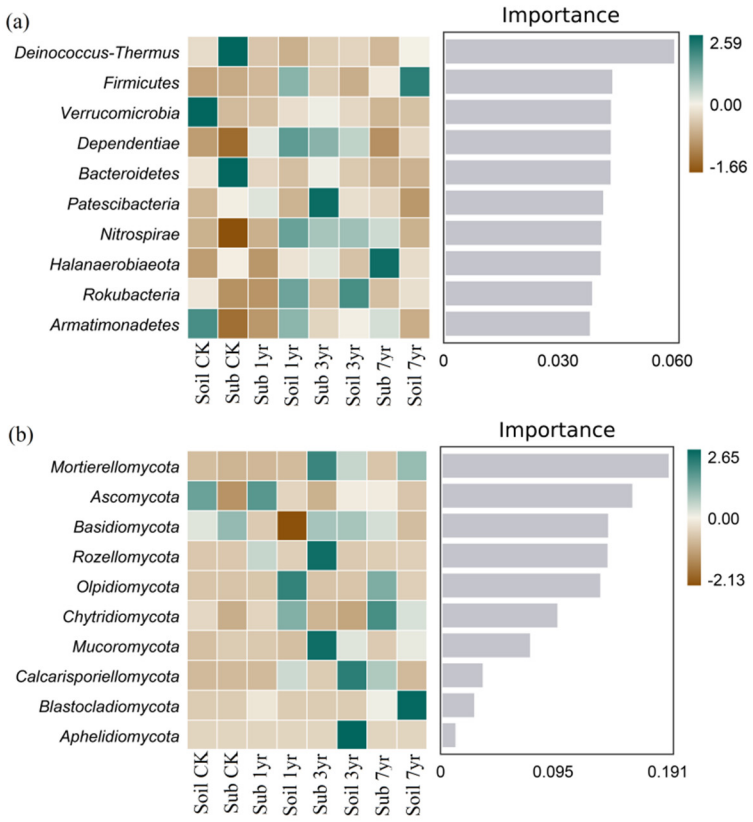

Figure 4. Random forests-based importance analysis of bacterial (**a**) and fungal (**b**) community composition of the top-10 most abundant phyla of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the abbreviations of the treatments.

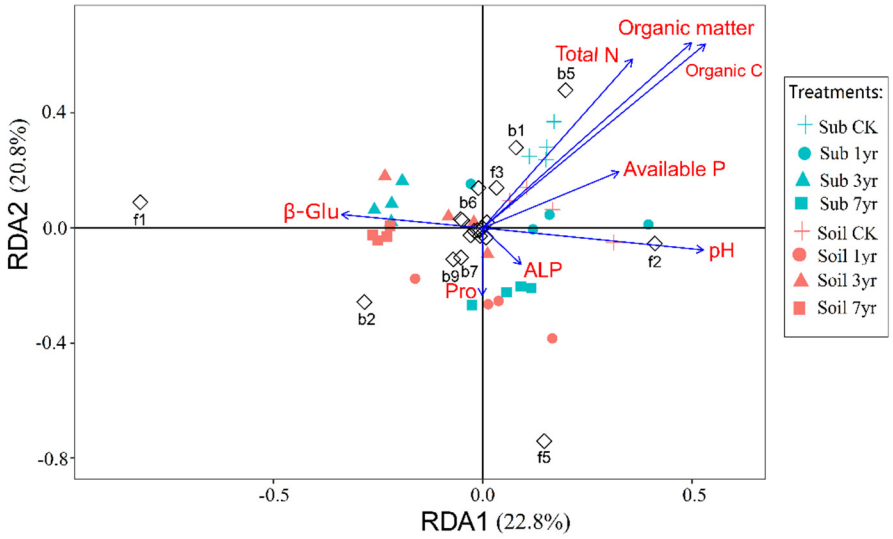

Figure 5. Redundancy analysis (RDA) of soil properties and dominant microbial taxa of different cultivation duration of the substrate and the surrounding soil. See Table S1 for the treatments. (Details: b1-b10 represent top 10 bacteria phyla, f1-f10 represents top 10 fungi phyla. b1, Deinococcus-Thermus; b2, Firmicutes; b3, Verrucomicrobia; b4, Dependentiae; b5, Bacteroidetes; b6, Patescibacteria; b7, Nitrospirae, b8, Halanaerobiaeota; b9, Rokubacteria; b10, Armatimonadetes; f1, Mortierellomycota; f2, Ascomycota; f3, Basidiomycota; f4, Rozellomycota; f5, Olpidiomycota; f6, Chytridiomycota; f7, Mucoromycota; f8, Calcarisporiellomycota; f9, Blastocladiomycota, f10, Aphelidiomycota.).

 doi:10.20944/preprints202307.1215.v1

4. Discussion

Spent mushroom compost (SMC) substrates, which comprise a recycled and reutilized waste products in mushroom cultivation, have been recognized as an organic material source and soil amendment for greenhouse vegetable farming [11]. In this experiment, we found that SMC substrates had more abundant nutrients than continuous mono-cropping soil (soil CK) and the soil surrounding the substrate (Table 1). Particularly, in the greenhouse the total N and total P contents were significantly higher in the SMC substrate than in the surrounding soil [57,58]. These results reinforced that the application of SMC substrates can provide a suitable growth medium for horticultural crops [59], and can enhance the crop yields in greenhouse farming [11]. Meanwhile, the physicochemical soil properties of the SMC substrate were significantly decreased along with the duration of cultivation (Table 1). It was proved that nutrient reduction and the depletion of the SMC substrate was accompanied by plant growth with longer cultivation duration [60]. Nevertheless, the physicochemical soil properties were significantly higher in the SMC substrate than in the surrounding soil (Table 1). Lou et al. [61] proved that the SMC substrate applied to agricultural land enhanced the soil organic matter and nutrient contents, while it reduced the total N leaching. These results demonstrated that the SMC substrate was an efficient alternative growth medium for the cultivation of horticultural crops compared to typical greenhouse soil.

Enzyme activities are an indicator of soil quality and participate in nutrient cycling during plant growth [62], which can be utilized to assess plant growth medium in ecosystems in response to available nutrients and metabolic requirements [63]. Soil alkaline phosphatases [64], soil protease [65], and β-Glucosidase [66] are important enzymes for available P, N, and C cycling processes. The concentrations of these enzymes differ between substrates and surrounding soils under various cultivation durations (Table 2), which is mainly due to differences in physicochemical soil properties, such as soil organic matter, total N, and pH, and soil enzyme activities involving Glu, ALP, and Pro enzymes (Table 1, Figure 5). Sinsabaugh [67] reported that extracellular enzymes are often associated with the acquisition, transformation, and mineralization of C, P, and N in their growth medium. Among the older cultivated plants, the activities of C, N, and P cycling enzymes were reduced in the substrate medium (Table 2). It was elucidated that crop growth and harvesting can lead to decreased enzyme activities due to the reduction/shortage of nutrients year by year in the growth medium [68]. The β-Glucosidase activity was enhanced with longer cultivation duration, whereas the activities of protease and alkaline phosphatases reached their peak values under the Soil 1yr and Soil 3yr treatments compared to the continuous mono-cropping soil (Soil CK treatment), respectively (Table 2). These results may help to explain how enzyme activities were influenced by crop root systems via the alteration of root system architectures [69] and rhizosphere exudations [70] in the growth medium, as well as the exchange of nutrients between the SMC substrate and surrounding soil. Furthermore, enzyme activities primarily originated from root secretion and microorganisms [71], and were closely correlated with microbial communities and structures in the growth media ecosystem [72].

Spent mushroom compost (SMC) substrates, consisting of spent mushroom compost, manure residues, and other agricultural waste, can partially or completely substitute growth media for horticultural crop production in greenhouses [73]. Meng et al. [74] reported that SMC substrates were a better alternative than peat-based growth media for greenhouse tomato and pepper seedlings due to higher morphological growth and lower instances of *Fusarium* (of *Ascomycota* phylum) pathogen infections. It is also known that SMC substrates can be employed as an alternative source of organic matter for crop growth, which contribute to increased soil microbiological activities [75]. In this study, following multiple years of tomato cultivation, the SMC substrates still had higher microbial alpha-diversity than continuous monocropping soil (Soil CK) and non-planted SMC substrates (Sub CK) treatment (Figure 1). This result confirmed that crop root systems can augment microbial diversity through the formation of beneficial symbionts [76,77]. Undergoing several years of tomato cultivation, the compositions of bacterial and fungal communities showed similar trend in the growth medium (substrate and surrounding soil) (Figure 2). It was revealed that continuous cropping practices altered the microbial community structures and compositions in the rhizospheric soil

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 18 July 2023 doi:10.20944/preprints202307.1215.v1

[23,78], as well as in the spent mushroom substrate [79]. The continuous cropping system is a common practice in greenhouse vegetable farming, which has adverse effects on horticultural crop yields and quality due to pathogenic diseases [80].

Continuous cropping obstacles (CCO) induce declines in crop quality and yields and the exacerbation of diseases and pests [81], which may be correlated with the modification of soil enzyme activities and microorganism communities [82]. A reduction in beneficial microbes (i.e *Bacillus* and *Trichoderma*) and accumulation of fungal pathogens *Fusarium* occurred under various continuous cropping practices in greenhouse soil [83]. *Fusarium* wilt (FW) in strawberry was mainly caused by *Fusarium oxysporum* fungal pathogens in greenhouses [84]. In this study, the continuous cropping soil of tomatoes in greenhouses (Soil CK) exhibited a higher abundance of pathogenic bacteria of the *Cyanobacteria* phyla and fungi of the *Ascomycota* phyla(Table 3, Figure S3). Denikina et al. [85] reported that *Cyanobacteria* taxa were associated with the diseased endemic sponge *Lubomirskia baicalensis* in Lake Baikal. Challacombe et al. [86] revealed that *Ascomycota* fungi can exist as latent saprotrophs or pathogens within plant tissues based on genome and secretome analyses in arid ecosystems. In contrast, the SMC substrates had a higher abundance of bacteria (*Deinococcus-Thermus, Patescibacteria, Dependentiae, Halanaerobiaeota,* and *Nitrospirae*) (Table 3, Figure 4a) and fungi (*Basidiomycota, Rozellomycota, Mortierellomycota, Mucoromycota, Chytridiomycota*, and *Olpidiomycota*) (Table 3, Figure 4b). *Deinococcus-Thermus* bacteria are highly resistant against extreme environmental stress [87,88]. Tian et al. [89] demonstrated that the bacteria super-phyla *Patescibacteria* contains ultra-small cells, simple membrane structures and streamlined redundant and nonessential functions to avoid phage predation, and adapt to specific stressed environments. It was reported that the *Halanaerobiaeota* phyla was dominant in extremely haloalkaline environments due to its salt tolerance and anaerobic attributes [90], while *Nitrospirae* phyla were less prevalent and participated in C and N cycling between different ecosystems [91,92]. Further, as an important ectomycorrhizal fungi, the *Basidiomycota* phyla can undergo symbiosis with host plants, which become their C sources and habitats [93]. Both *Rozellomycota* and *Chytridiomycota* fungi belong to the Zoosporic phyla with motile spores, which typically play critical ecological roles in the recycling of energy and matter in food webs [94]. As fast growing saprotrophic fungi, the *Mortierellomycota* phyla are potentially influenced by soil temperature [95] and have important biological functions for the protection of plants against pathogens [96]. Interestingly, *Mucoromycota* fungi may be utilized as a biorefinery that employs fungi for its highly versatile metabolic system, which can generate several valuable bioproducts including of pigments, polyphosphates, ethanol, organic acids, enzymes, as well as low- and high-value lipids [97,98]. Therefore, it was revealed in this study that the greenhouse SMC substrate model was more enriched with several beneficial microbes than the continuous cropping field model.

Compared with open-field cultivation, plastic-greenhouse cultivation is a popular agricultural production platform on a global scale [9]. With the advantages of a prolonged growing season with a stable hospitable environment, plastic-greenhouse cultivation is preferred in many cases for the production of high value vegetables and other crops [99,100]. However, long-term intensive plastic-greenhouse cultivation can easily give rise to continuous cropping obstacles (CCO), which lead to deleterious changes in the composition of soil microbial communities [9]. Interesting, this study suggested that the long-term intensive cropping of tomatoes with the SMC substrate growth medium (1-, 3-, and 7-years cultivation) in a plastic-greenhouse had more similar bacterial beta-diversity than the non-planted SMC substrates (Sub CK) (Figure 2). It was demonstrated that long-term greenhouse vegetable cultivation with the SMC substrate growth medium altered the structures of soil microbial communities, as reported by Liu et al. [101]. Furthermore, the soil fungi beta-diversity of surrounding soil between the different cultivation durations (1-, 3-, and 7-years cultivation) was relatively consistent, in contrast to continuous mono-cropping soil (Soil CK) (Figure 2b). The root systems [102] and root exudates [103] of vegetable crops play critical roles in the compositional restructuring of soil microbial communities in the rhizosphere and surrounding soil environments, particularly under continuous mono-cropping [104]. Unal [11] suggested that SMC substrates were a viable alternative growth media for tomato seedling production in plastic greenhouses. Thus, SMC substrate

cultivation might be a feasible strategy for the improved large scale production of horticultural vegetables and crops in greenhouses worldwide [73].

Meanwhile, we found that the dominant taxa *Dongia* and *Sphingomonas* (*Proteobacteria* phyla) were highly abundant in the continuous mono-cropping soil (Soil CK) (Figure 3a). Pathogenic bacteria in the *Sphingomonas* genus commonly cause brown spot disease on yellow Spanish melon fruits [105], and rase Panax ginseng with rusty root disease that seriously affects its production [106]. Yet, several beneficial bacterial genera (i.e. *Saccharimonadales*, *Nocardioides*, *Gaiella*) were significantly dominant in the SMC substrate growth media for tomato cultivation in the greenhouse (Figure 3a). *Saccharimonadales* (*Patescibacteria* phylum) was influenced by soil sugar concentrations, the high abundance of which can enhance soil alkaline phosphatase activities [107]. *Nocardioides* (*Actinobacteria* phylum) was responsible for the degradation of vinyl chloride (VC), which is carcinogenic to humans [108]. Zhao et al. [109] reported that *Nocardioides* and *Gaiella* were important beneficial bacteria for the suppression of *Fusarium* wilt in a long-term tomato monoculture soil. Even the *Bacillus* genus of important beneficial bacteria exhibited a significantly higher abundance in the surrounding soil of the 7-year cultivation (Figure 3a) [83]. Fungi were enriched in the SMC substrate and surrounding soil environment, which included several potentially beneficial fungal taxa of *Thermomyces* that secrete glycoside hydrolase and proteases [110] and coprophilous fungi of *Kernia* genus [111]. Further, the biodegradation fungi of the *Kernia* and *Mortierella* genera [112], the core fungus of *Remersonia* genus involved in humification processes [113], bioremediation microbiomes of contaminated *Aspergillus* genus environments [114], and alkaliphilic fungus of *Sodiomyces* genus were present [115], (Figure 3b). In contrast, the *Ascobolus* [116] and *Fusarium* [117] taxa of potential pathogens were significantly decreased during long-term cultivation with the SMC growth medium under greenhouse conditions.

5. Conclusions

The application of agro-based spent mushroom compost (SMC) substrates using a groove model system in greenhouses is a novel approach for the cultivation of horticultural plants. It was concluded that as an alternative growth media, SMC substrates could enhance the concentrations of major nutrients, such as total N and total P. In conjunction with longer cultivation durations, the physicochemical soil properties and soil enzyme activities were significantly reduced. However, the microbial alpha-diversity was considerably higher in the growth media of the groove model regardless of cultivation duration, in contrast to the continuous monocropping soil (Soil CK) and non-planted SMC substrate (Sub CK) treatment. The composition of bacterial communities had more similarities than fungal communities in both the SMC growth substrate and surrounding soil environment along with longer duration cultivation. Compared with the greenhouse continuous monocropping soil (Soil CK), the SMC substrate recruited more beneficial microbes, such as bacteria (*Deinococcus-Thermus, Halanaerobiaeota,* and *Nitrospirae* phyla) and fungi (*Basidiomycota, Mortierellomycota,* and *Chytridiomycota* phyla). Several potential beneficial bacterial genera (*Saccharimonadales, Gaiella, Bacillus*) and fungal genera (*Thermomyces, Kernia*, and *Mortierella*) were enriched in both the SMC substrate and surrounding soil. In general, the application of agro-based SMC substrates is recommended as a suitable growth media in the groove model for horticulture plant production under greenhouse conditions in North-western China.

Supplementary Materials: Nucleotide sequence data[The nucleotide data has been submitted and deposited to the SRA (Sequence Read Archive) at the National Center for Biotechnology Information (NCBI) under accession number SUB12292980 (biosample SAMN31710080) for 16S bacteria sequences and number SUB12293105 (biosample SAMN31710019) for ITS fungi sequences. The review links as below: https://dataview.ncbi.nlm.nih.gov/object/PRJNA901255?reviewer=9n9qumsq784i9klabfct48k0db.https://dataview.ncbi.nlm.nih.gov/object/PRJNA901255?reviewer=9n9qumsq784i9klabfct48k0db]: The nucleotide data has been submitted and deposited in the GenBank databases.

Author Contributions: C.G.H. and Y.J.N. Conceptualization; C.G.H., Y.J.C., X.L.H., M.K., M.M.T., Y.Y.F., X.L.L. and Q.L. Methodology, Data collection; C.G.H. and X.L.H. Data analysis, Writing-Original draft preparation; M.K. Supervision; Q.L. Software, Validation; Y.L.C. Writing-Reviewing and Editing.

Funding: This work was supported by Shanxi Province Scientific and Technological Achievements Transformation guidance special project of China [grant numbers: 201904D131038; 202104021301045].

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Smith, G. R., and Archer, R. (2020). Climate, population, food security: adapting and evolving in times of global change. *International Journal of Sustainable Development and World Ecology* **27**, 419-423.
2. Abbade, E. B., and Dewes, H. (2015). Food insecurity worldwide derived from food supply patterns. *Food Security* **7**, 109-120.
3. Schmitt, J., Offermann, F., Söder, M., Frühauf, C., and Finger, R. (2022). Extreme weather events cause significant crop yield losses at the farm level in German agriculture. *Food Policy* **112**, 102359.
4. Shen, J., Zhang, P., Chang, Y., Zhang, L., Hao, Y., Tang, S., and Xiong, X. (2021). The environmental performance of greenhouse versus open-field cherry production systems in China. *Sustainable Production and Consumption* **28**, 736-748.
5. Kumar, K. S., Jha, M. K., Tiwari, K. N., and Singh, A. (2010). Modeling and evaluation of greenhouse for floriculture in subtropics. *Energy and Buildings* **42**, 1075-1083.
6. Iddio, E., Wang, L., Thomas, Y., McMorrow, G., and Denzer, A. (2020). Energy efficient operation and modeling for greenhouses: A literature review. *Renewable & Sustainable Energy Reviews* **117**.
7. Yu, H. Y., Li, T. X., and Zhang, X. Z. (2010). Nutrient Budget and Soil Nutrient Status in Greenhouse System. *Agricultural Sciences in China* **9**, 871-879.
8. Hong, E. M., Choi, J. Y., Nam, W. H., Kang, M. S., and Jang, J. R. (2014). Monitoring nutrient accumulation and leaching in plastic greenhouse cultivation. *Agricultural Water Management* **146**, 11-23.
9. Liu, X., Li, Y., Ren, X., Chen, B., Zhang, Y., Shen, C., Wang, F., and Wu, D. (2020). Long-Term Greenhouse Cucumber Production Alters Soil Bacterial Community Structure. *Journal of Soil Science and Plant Nutrition* **20**, 306-321.
10. Sun, H., Wei, C., Xu, W., Yang, J., Wang, X., and Qiu, Y. (2019). Characteristics of salt contents in soils under greenhouse conditions in China. *Environmental Science and Pollution Research* **26**, 3882-3892.
11. Polat, E., Uzun, H. I., Topcuoglu, B., Oenal, K., Onus, A. N., and Karaca, M. (2009). Effects of spent mushroom compost on quality and productivity of cucumber (Cucumis sativus L.) grown in greenhouses. *African Journal of Biotechnology* **8**, 176-180.
12. Unal, M. (2015). The utilization of spent mushroom compost applied at different rates in tomato (Lycopersicon esculentum Mill.) seedling production. *Emirates Journal of Food and Agriculture* **27**, 692-697.
13. Meng, L. Q., Li, W. G., Zhang, X. C., Zhao, Y., Chen, L., and Zhang, S. M. (2020). Influence of spent mushroom substrate and molasses amendment on nitrogen loss and humification in sewage sludge composting. *Heliyon* **6**.
14. Najafi, B., Ardabili, S. F., Shamshirband, S., and Chau, K.-w. (2019). Spent mushroom compost (SMC) as a source for biogas production in Iran. *Engineering Applications of Computational Fluid Mechanics* **13**, 967-982.
15. Leong, Y. K., Ma, T.-W., Chang, J.-S., and Yang, F.-C. (2022). Recent advances and future directions on the valorization of spent mushroom substrate (SMS): A review. *Bioresource Technology* **344**, 126157.
16. Szulc, W., Rutkowska, B., Kusmirek, E., Spychaj-Fabisiak, E., Kowalczyk, A., and Debska, K. (2019). Yielding, Chemcial Composition and Nitrogen Use Efficiency Determined for White Cabbage (*Brassica Oleracea* L. Var. Capitata L.) Supplied Organo-mineral Fertilizers From Spent Mushroom Substrate. *Journal of Elementology* **24**, 1063-1077.
17. Marin-Benito, J. M., Sanchez-Martin, M. J., and Rodriguez-Cruz, M. S. (2016). Impact of Spent Mushroom Substrates on the Fate of Pesticides in Soil, and Their Use for Preventing and/or Controlling Soil and Water Contamination: A Review. *Toxics* **4**.
18. Alves, M. J., Ferreira, I. C. F. R., Dias, J., Teixeira, V., Martins, A., and Pintado, M. (2012). A Review on Antimicrobial Activity of Mushroom (Basidiomycetes) Extracts and Isolated Compounds. *Planta Medica* **78**, 1707-1718.
19. Ngan, N. M., and Riddech, N. (2021). Use of Spent Mushroom Substrate as an Inoculant Carrier and an Organic Fertilizer and Their Impacts on Roselle Growth (Hibiscus sabdariffa L.) and Soil Quality. *Waste and Biomass Valorization* **12**, 3801-3811.

20. Ge, T., Nie, S. a., Wu, J., Shen, J., Xiao, H. a., Tong, C., Huang, D., Hong, Y., and Iwasaki, K. (2011). Chemical properties, microbial biomass, and activity differ between soils of organic and conventional horticultural systems under greenhouse and open field management: a case study. *Journal of Soils and Sediments* **11**, 25-36.
21. Hao, Z., Chen, B., and Li, X. (2020). Relationship between soil chemical properties and microbial metabolic patterns in intensive greenhouse tomato production systems. *Archives of Agronomy and Soil Science* **66**, 1334-1343.
22. Tong, L., Zhu, L., Lv, Y., Zhu, K., Liu, X., and Zhao, R. (2020). Response of organic carbon fractions and microbial community composition of soil aggregates to long-term fertilizations in an intensive greenhouse system. *Journal of Soils and Sediments* **20**, 641-652.
23. Li, Y., Chi, J., Ao, J., Gao, X., Liu, X., Sun, Y., and Zhu, W. (2021). Effects of Different Continuous Cropping Years on Bacterial Community and Diversity of Cucumber Rhizosphere Soil in Solar-Greenhouse. *Current Microbiology* **78**, 2380-2390.
24. Ahmad, I., Ahmad, T., Gulfam, A., and Saleem, M. (2012). Growth and Flowering of Gerbera as Influenced by Various Horticultural Substrates. *Pakistan Journal of Botany* **44**, 291-299.
25. Gonani, Z., Riahi, H., and Sharifi, K. (2011). Impact of Using Leached Spent Mushroom Compost as a Partial Growing Media for Horticultural Plants. *Journal of Plant Nutrition* **34**, 337-344.
26. Thomas, G. (1996). Soil pH and soil acidity. *Methods of Soil Analysis Part 3—Chemical Methods*, 475-490.
27. Cao, N., Chen, X., Cui, Z., and Zhang, F. (2012). Change in soil available phosphorus in relation to the phosphorus budget in China. *Nutrient Cycling in Agroecosystems* **94**, 161-170.
28. Guo, W., Chen, H., and Zhang, Q. (2011). Effects of biochar application on total nitrogen and alkali-hydrolyzable nitrogen content in the topsoil of the high-yield cropland in north China Plain. *Ecology & Environmental Sciences*.
29. Ryan, J., Estefan, G., and Rashid, A. (2007). "Soil and plant analysis laboratory manual," ICARDA.
30. Zhou, X. Q., Wu, H. W., Li, G. D., and Chen, C. R. (2016). Short-term contributions of cover crop surface residue return to soil carbon and nitrogen contents in temperate Australia. *Environmental Science and Pollution Research* **23**, 23175-23183.
31. Li, W.-h., Zhang, C.-b., Jiang, H.-b., Xin, G.-r., and Yang, Z.-y. (2006). Changes in soil microbial community associated with invasion of the exotic weed, Mikania micrantha HBK. *Plant and Soil* **281**, 309-324.
32. Castro-Jimenez, J., and Gonzalez, C. (2011). Immunoassay-based screening of polychlorinated biphenyls (PCB) in sediments: requirements for a new generation of test kits. *Journal of Environmental Monitoring* **13**, 894-900.
33. Davinic, M., Fultz, L. M., Acosta-Martinez, V., Calderon, F. J., Cox, S. B., Dowd, S. E., Allen, V. G., Zak, J. C., and Moore-Kucera, J. (2012). Pyrosequencing and mid-infrared spectroscopy reveal distinct aggregate stratification of soil bacterial communities and organic matter composition. *Soil Biology & Biochemistry* **46**, 63-72.
34. Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2 (vol 37, pg 852, 2019). *Nature Biotechnology* **37**, 1091-1091.
35. Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *Embnet Journal* **17**.
36. Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**, 581-+.
37. Katoh, K., Misawa, K., Kuma, K.-i., and Miyata, T. (2002). MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic acids research* **30**, 3059-66.
38. Price, M. N., Dehal, P. S., and Arkin, A. P. (2009). FastTree: Computing Large Minimum Evolution Trees with Profiles instead of a Distance Matrix. *Molecular Biology and Evolution* **26**, 1641-1650.
39. Chao, A. (1984). Nonparametric estimation of the number of classes in a population. *Scand. J. Statist* **11**, 265-270.
40. Shannon, C. E. (1948). A mathematical theory of communication. *The Bell System Technical Journal* **27**, 379-423.
41. Lozupone, C. A., Hamady, M., Kelley, S. T., and Knight, R. (2007). Quantitative and qualitative beta diversity measures lead to different insights into factors that structure microbial communities. *Applied and environmental microbiology* **73**, 1576-85.

42. Lozupone, C., and Knight, R. (2005). UniFrac: a new phylogenetic method for comparing microbial communities. *Applied and environmental microbiology* **71**, 8228-35.
43. Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., and Caporaso, J. G. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2 ' s q2-feature-classifier plugin. *Microbiome* **6**, 90-98.
44. Koljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., and Bahram, M., et al. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology* **22**, 5271-5277.
45. Bray, J. R., and Curtis, J. T. (1957). An Ordination of the Upland Forest Communities of Southern Wisconsin. *Ecological Monographs* **27**.
46. Ramette, A. (2007). Multivariate analyses in microbial ecology. *FEMS microbiology ecology* **62**, 142-60.
47. Brian, H., McArdle, Marti, J., and Anderson (2001). Fitting multivariate models to community data: a comment on distanceâbased redundancy analysis. *Ecology* **82**, 290-297.
48. Warton, D. I., Wright, S. T., and Wang, Y. (2012). Distance-based multivariate analyses confound location and dispersion effects. *Methods in Ecology and Evolution* **3**, 89-101.
49. Anderson, M. J., Ellingsen, K. E., and McArdle, B. H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology letters* **9**, 683-93.
50. Huson, D. H., Mitra, S., Ruscheweyh, H. J., Weber, N., and Schuster, S. C. (2011). Integrative analysis of environmental sequences using MEGAN4. *Genome Research* **21**, 1552-1560.
51. Asnicar, F., Weingart, G., Tickle, T. L., Huttenhower, C., and Segata, N. (2015). Compact graphical representation of phylogenetic data and metadata with GraPhlAn. *Peerj* **3**.
52. Zaura, E., Keijser, B. J. F., Huse, S. M., and Crielaard, W. (2009). Defining the healthy "core microbiome" of oral microbial communities. *Bmc Microbiology* **9**.
53. Zgadzaj, R., Garrido-Oter, R., Jensen, D. B., Koprivova, A., Schulze-Lefert, P., and Radutoiu, S. (2016). Root nodule symbiosis in Lotus japonicus drives the establishment of distinctive rhizosphere, root, and nodule bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* **113**, E7996-E8005.
54. Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., and Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology* **12**.
55. Bai, J., Li, Y., Li, J., Yang, X., Jiang, Y., and Xia, S.-T. (2022). Multinomial random forest. *Pattern Recognition* **122**, 108331.
56. Breiman (2001). Random forests. *Mach Learn* **45**, 5-32.
57. Hanafi, F. H. M., Rezania, S., Taib, S. M., Din, M. F. M., Yamauchi, M., Sakamoto, M., Hara, H., Park, J., and Ebrahimi, S. S. (2018). Environmentally sustainable applications of agro-based spent mushroom substrate (SMS): an overview. *Journal of Material Cycles and Waste Management* **20**, 1383-1396.
58. Lou, Z., Zhu, J., Wang, Z., Baig, S. A., Fang, L., Hu, B., and Xu, X. (2015). Release characteristics and control of nitrogen, phosphate, organic matter from spent mushroom compost amended soil in a column experiment. *Process Safety and Environmental Protection* **98**, 417-423.
59. Gumus, I., and Seker, C. (2017). Effects of spent mushroom compost application on the physicochemical properties of a degraded soil. *Solid Earth* **8**, 1153-1160.
60. Henry, A., Chaves, N. F., Kleinman, P. J. A., and Lynch, J. P. (2010). Will nutrient-efficient genotypes mine the soil? Effects of genetic differences in root architecture in common bean (Phaseolus vulgaris L.) on soil phosphorus depletion in a low-input agro-ecosystem in Central America. *Field Crops Research* **115**, 67-78.
61. Lou, Z., Sun, Y., Bian, S., Ali Baig, S., Hu, B., and Xu, X. (2017). Nutrient conservation during spent mushroom compost application using spent mushroom substrate derived biochar. *Chemosphere* **169**, 23-31.
62. Adetunji, A. T., Lewu, F. B., Mulidzi, R., and Ncube, B. (2017). The biological activities of beta-glucosidase, phosphatase and urease as soil quality indicators: a review. *Journal of Soil Science and Plant Nutrition* **17**, 794-807.
63. Caldwell, B. A. (2005). Enzyme activities as a component of soil biodiversity: a review. *Pedobiologia* **49**, 637-644.
64. Nannipieri, P., Giagnoni, L., Landi, L., and Renella, G. (2011). Role of phosphatase enzymes in soil. *In* "Phosphorus in action", pp. 215-243. Springer.
65. Vranova, V., Rejsek, K., and Formanek, P. (2013). Proteolytic activity in soil: A review. *Applied Soil Ecology* **70**, 23-32.

66. Utobo, E. B., and Tewari, L. (2015). Soil Enzymes as Bioindicators of Soil Ecosystme Status. *Applied Ecology and Environmental Research* **13**, 147-169.
67. Sinsabaugh, R. L. (2010). Phenol oxidase, peroxidase and organic matter dynamics of soil. *Soil Biology & Biochemistry* **42**, 391-404.
68. Luan, H. A., Gao, W., Tang, J. W., Li, R. N., Li, M. Y., Zhang, H. Z., Chen, X. P., Masiliunas, D., and Huang, S. W. (2020). Aggregate-associated changes in nutrient properties, microbial community and functions in a greenhouse vegetable field based on an eight-year fertilization experiment of China. *Journal of Integrative Agriculture* **19**, 2530-2548.
69. Yahya, M., ul Islam, E., Rasul, M., Farooq, I., Mahreen, N., Tawab, A., Irfan, M., Rajput, L., Amin, I., and Yasmin, S. (2021). Differential Root Exudation and Architecture for Improved Growth of Wheat Mediated by Phosphate Solubilizing Bacteria. *Frontiers in Microbiology* **12**.
70. Zhang, X., Dippold, M. A., Kuzyakov, Y., and Razavi, B. S. (2019). Spatial pattern of enzyme activities depends on root exudate composition. *Soil Biology & Biochemistry* **133**, 83-93.
71. Bilyera, N., Zhang, X., Duddek, P., Fan, L., Banfield, C. C., Schluter, S., Carminati, A., Kaestner, A., Ahmed, M. A., Kuzyakov, Y., Dippold, M. A., Spielvogel, S., and Razavi, B. S. (2021). Maize genotype-specific exudation strategies: An adaptive mechanism to increase microbial activity in the rhizosphere. *Soil Biology & Biochemistry* **162**.
72. Luo, S.-p., He, B.-h., Zeng, Q.-p., Li, N.-j., and Yang, L. (2020). Effects of seasonal variation on soil microbial community structure and enzyme activity in a Masson pine forest in Southwest China. *Journal of Mountain Science* **17**, 1398-1409.
73. Zied, D. C., de Abreu, C. G., Alves, L. d. S., Prado, E. P., Pardo-Gimenez, A., de Melo, P. C., and Dias, E. S. (2021). Influence of the production environment on the cultivation of lettuce and arugula with spent mushroom substrate. *Journal of Environmental Management* **281**.
74. Meng, X., Dai, J., Zhang, Y., Wang, X., Zhu, W., Yuan, X., Yuan, H., and Cui, Z. (2018). Composted biogas residue and spent mushroom substrate as a growth medium for tomato and pepper seedlings. *Journal of Environmental Management* **216**, 62-69.
75. Zhang, L., and Sun, X. (2014). Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar. *Bioresource Technology* **171**, 274-284.
76. Huang, C., Yang, W., Chen, Y., Han, X., Khan, S., Gao, Z., and Yang, Z. (2020). Ridge-furrow and film-mulching sowing practices enhance enzyme activity and alter fungi communities. *Agronomy Journal* **112**, 4775-4787.
77. Miras-Avalos, J. M., Antunes, P. M., Koch, A., Khosla, K., Klironomos, J. N., and Dunfield, K. E. (2011). The influence of tillage on the structure of rhizosphere and root-associated arbuscular mycorrhizal fungal communities. *Pedobiologia* **54**, 235-241.
78. Li, J., Chen, X., Li, S., Zuo, Z., Zhan, R., and He, R. (2020). Variations of rhizospheric soil microbial communities in response to continuous Andrographis paniculata cropping practices. *Botanical Studies* **61**, 18.
79. Wang, H.-W., Xu, M., Cai, X.-Y., and Tian, F. (2021). Evaluation of soil microbial communities and enzyme activities in cucumber continuous cropping soil treated with spent mushroom (Flammulina velutipes) substrate. *Journal of Soils and Sediments* **21**, 2938-2951.
80. Sun, K., Fu, L., Song, Y., Yuan, L., Zhang, H., Wen, D., Yang, N., Wang, X., Yue, Y., Li, X., and Wang, K. (2021). Effects of continuous cucumber cropping on crop quality and soil fungal community. *Environmental Monitoring and Assessment* **193**, 436.
81. Zhao, Y., Qin, X. M., Tian, X. P., Yang, T., Deng, R., and Huang, J. (2021). Effects of continuous cropping of Pinellia ternata (Thunb.) Breit. on soil physicochemical properties, enzyme activities, microbial communities and functional genes. *Chemical and Biological Technologies in Agriculture* **8**, 43.
82. Gao, Z., Hu, Y., Han, M., Xu, J., Wang, X., Liu, L., Tang, Z., Jiao, W., Jin, R., Liu, M., Guan, Z., and Ma, Z. (2021). Effects of continuous cropping of sweet potatoes on the bacterial community structure in rhizospheric soil. *BMC Microbiology* **21**, 102.
83. Huang, Y., Xiao, X., Huang, H., Jing, J., Zhao, H., Wang, L., and Long, X.-E. (2018). Contrasting beneficial and pathogenic microbial communities across consecutive cropping fields of greenhouse strawberry. *Applied Microbiology and Biotechnology* **102**, 5717-5729.

84. Dinler, H., Benlioglu, S., and Benlioglu, K. (2016). Occurrence of Fusarium wilt caused by Fusarium oxysporum on strawberry transplants in Aydın Province in Turkey. *Australasian Plant Disease Notes* **11**, 10.
85. Denikina, N. N., Dzyuba, E. V., Bel'kova, N. L., Khanaev, I. V., Feranchuk, S. I., Makarov, M. M., Granin, N. G., and Belikov, S. I. (2016). The first case of disease of the sponge Lubomirskia baicalensis: Investigation of its microbiome. *Biology Bulletin* **43**, 263-270.
86. Challacombe, J. F., Hesse, C. N., Bramer, L. M., McCue, L. A., Lipton, M., Purvine, S., Nicora, C., Gallegos-Graves, L. V., Porras-Alfaro, A., and Kuske, C. R. (2019). Genomes and secretomes of Ascomycota fungi reveal diverse functions in plant biomass decomposition and pathogenesis. *BMC Genomics* **20**, 976.
87. Mariano, C., Mello, I. S., Barros, B. M., da Silva, G. F., Terezo, A. J., and Soares, M. A. (2020). Mercury alters the rhizobacterial community in Brazilian wetlands and it can be bioremediated by the plant-bacteria association. *Environmental Science and Pollution Research* **27**, 13550-13564.
88. White, O., Eisen, J. A., Heidelberg, J. F., Hickey, E. K., Peterson, J. D., and Dodson, R. J., et al. (1999). Genome Sequence of the Radioresistant Bacterium Deinococcus radiodurans R1. *Science* **286**, 1571-1577.
89. Tian, R., Ning, D., He, Z., Zhang, P., Spencer, S. J., and Gao, S., et al. (2020). Small and mighty: adaptation of superphylum Patescibacteria to groundwater environment drives their genome simplicity. *Microbiome* **8**, 51.
90. Li, X., Yang, M., Mu, T., Miao, D., Liu, J., and Xing, J. (2022). Composition and key-influencing factors of bacterial communities active in sulfur cycling of soda lake sediments. *Archives of Microbiology* **204**, 317.
91. Abujabhah, I. S., Doyle, R. B., Bound, S. A., and Bowman, J. P. (2018). Assessment of bacterial community composition, methanotrophic and nitrogen-cycling bacteria in three soils with different biochar application rates. *Journal of Soils and Sediments* **18**, 148-158.
92. Baker, B. J., Sheik, C. S., Taylor, C. A., Jain, S., Bhasi, A., Cavalcoli, J. D., and Dick, G. J. (2013). Community transcriptomic assembly reveals microbes that contribute to deep-sea carbon and nitrogen cycling. *The ISME Journal* **7**, 1962-1973.
93. Luptáková, E., and Mihál, I. (2020). Dynamics of ectomycorrhizal mycobiota (Basidiomycota) communities on a former agricultural land (West Carpathians). *Mycological Progress* **19**, 845-857.
94. Hurdeal, V. G., Gentekaki, E., Hyde, K. D., and Jeewon, R. (2021). Where are the basal fungi? Current status on diversity, ecology, evolution, and taxonomy. *Biologia* **76**, 421-440.
95. Dahl, M. B., Peršoh, D., Jentsch, A., and Kreyling, J. (2021). Root-Associated Mycobiomes of Common Temperate Plants (Calluna vulgaris and Holcus lanatus) Are Strongly Affected by Winter Climate Conditions. *Microbial Ecology* **82**, 403-415.
96. Ghabrial, S. A., and Suzuki, N. (2009). Viruses of plant pathogenic fungi. *Annual review of phytopathology* **47**, 353-384.
97. Dzurendova, S., Losada, C. B., Dupuy-Galet, B. X., Fjær, K., and Shapaval, V. (2022). Mucoromycota fungi as powerful cell factories for modern biorefinery. *Applied Microbiology and Biotechnology* **106**, 101-115.
98. Kosa, G., Zimmermann, B., Kohler, A., Ekeberg, D., Afseth, N. K., Mounier, J., and Shapaval, V. (2018). High-throughput screening of Mucoromycota fungi for production of low- and high-value lipids. *Biotechnology for Biofuels* **11**, 66.
99. Ghannem, A., Ben Aissa, I., and Majdoub, R. (2021). Effects of regulated deficit irrigation applied at different growth stages of greenhouse grown tomato on substrate moisture, yield, fruit quality, and physiological traits. *Environmental science and pollution research international* **28**, 46553-46564.
100. Zhang, Q., Zhang, X., Yang, Z., Huang, Q., and Qiu, R. (2022). Characteristics of Plastic Greenhouse High-Temperature and High-Humidity Events and Their Impacts on Facility Tomatoes Growth. *Frontiers in Earth Science* **10**.
101. Liu, X., Zhang, Y., Ren, X., Chen, B., Shen, C., and Wang, F. (2019). Long-term greenhouse vegetable cultivation alters the community structures of soil ammonia oxidizers. *Journal of Soils and Sediments* **19**, 883-902.
102. Lareen, A., Burton, F., and Schäfer, P. (2016). Plant root-microbe communication in shaping root microbiomes. *Plant Molecular Biology* **90**, 575-587.
103. Bertin, C., Yang, X., and Weston, L. A. (2003). The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* **256**, 67-83.
104. Zhao, Y., Mao, X., Zhang, M., Yang, W., Di, H. J., Ma, L., Liu, W., and Li, B. (2020). Response of soil microbial communities to continuously mono-cropped cucumber under greenhouse conditions in a calcareous soil of north China. *Journal of Soils and Sediments* **20**, 2446-2459.

105. Buonaurio, R., Stravato, V. M., Kosako, Y., Fujiwara, N., Naka, T., Kobayashi, K., Cappelli, C., and Yabuuchi, E. (2002). Sphingomonas melonis sp. nov., a novel pathogen that causes brown spots on yellow Spanish melon fruits. *International journal of systematic and evolutionary microbiology* **52**, 2081-2087.
106. Kim, Y.-J., Park, J. Y., Balusamy, S. R., Huo, Y., Nong, L. K., Le, H. T., Yang, D. C., and Kim, D. (2020). Comprehensive Genome Analysis on the Novel Species Sphingomonas panacis DCY99(T) Reveals Insights into Iron Tolerance of Ginseng. *International Journal of Molecular Sciences* **21**.
107. Wang, G., Jin, Z., Wang, X., George, T. S., Feng, G., and Zhang, L. (2022). Simulated root exudates stimulate the abundance of Saccharimonadales to improve the alkaline phosphatase activity in maize rhizosphere. *Applied Soil Ecology* **170**.
108. Wilson, F. P., Liu, X., Mattes, T. E., and Cupples, A. M. (2016). Nocardioides, Sediminibacterium, Aquabacterium, Variovorax, and Pseudomonas linked to carbon uptake during aerobic vinyl chloride biodegradation. *Environmental Science and Pollution Research* **23**, 19062-19070.
109. Zhao, F. Y., Zhang, Y. Y., Dong, W. G., Zhang, Y. Q., Zhang, G. X., Sun, Z. P., and Yang, L. J. (2019). Vermicompost can suppress Fusarium oxysporum f. sp. lycopersici via generation of beneficial bacteria in a long-term tomato monoculture soil. *Plant and Soil* **440**, 491-505.
110. Shi, Z., Gong, W., Zhang, L., Dai, L., Chen, G., and Wang, L. (2019). Integrated Functional-Omics Analysis of Thermomyces lanuginosus Reveals its Potential for Simultaneous Production of Xylanase and Substituted Xylooligosaccharides. *Applied Biochemistry and Biotechnology* **187**, 1515-1538.
111. Caretta, G., Piontelli, E., Savino, E., and Bulgheroni, A. (1998). Some coprophilous fungi from Kenya. *Mycopathologia* **142**, 125-34.
112. Li, X. J., Li, Y., Zhao, X. D., Zhang, X. L., Zhao, Q., Wang, X., and Li, Y. T. (2019). Restructured fungal community diversity and biological interactions promote metolachlor biodegradation in soil microbial fuel cells. *Chemosphere* **221**, 735-749.
113. Kong, Z., Wang, M., Shi, X., Wang, X., Zhang, X., Chai, L., Liu, D., and Shen, Q. (2022). The functions of potential intermediates and fungal communities involved in the humus formation of different materials at the thermophilic phase. *Bioresource Technology* **354**.
114. Kour, D., Kaur, T., Devi, R., Yadav, A., Singh, M., Joshi, D., Singh, J., Suyal, D. C., Kumar, A., Rajput, V. D., Yadav, A. N., Singh, K., Singh, J., Sayyed, R. Z., Arora, N. K., and Saxena, A. K. (2021). Beneficial microbiomes for bioremediation of diverse contaminated environments for environmental sustainability: present status and future challenges. *Environmental Science and Pollution Research* **28**, 24917-24939.
115. Kozlova, M. V., Ianutsevich, E. A., Danilova, O. A., Kamzolkina, O. V., and Tereshina, V. M. (2019). Lipids and soluble carbohydrates in the mycelium and ascomata of alkaliphilic fungus Sodiomyces alkalinus. *Extremophiles* **23**, 487-494.
116. Xiang, D., Wu, Y., Li, H., Liu, Q., Zhou, Z. F., Chen, Q. H., Zhang, N., and Xu, L. (2021). Soil Fungal Diversity and Community Composition in Response to Continuous Sweet Potato Cropping Practices. *Phyton-International Journal of Experimental Botany* **90**, 1247-1258.
117. Rodríguez Zafra, J. M., de Cara García, M., Tello Marquina, J., and Palmero Llamas, D. (2016). Dispersal of Fusarium spp. by rainwater and pathogenicity on four plant species. *Aerobiologia* **32**, 431-439.