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Effect of vegetable residues incorporation on soil fertility, rhizosphere microbial community structure, and plant growth of continuously cropped cucumber in a solar greenhouse

Xiaolu Chen[1†], Dalong Zhang[1,2†], Xiaoya Li[1], Xiaotian Li[1], Jie Lou[1] and Min Wei[1,2,3*]

Abstract

Purpose: Incorporating crop residues into the soil is considered a sustainable and valuable method to alleviate soil deterioration caused by continuous monoculture in greenhouse production. However, the effect of vegetable residues retention on soil amendments is poorly understood. In the present study, we investigated the impacts of sweet pepper, tomato, and cucumber plant residues on soil microbial communities and plant growth of continuously cropped cucumber in a solar greenhouse.

Methods: The 16S rRNA and ITS1 rRNA genes were amplified, and high-throughput sequencing was performed to explore the impacts of vegetable residues incorporation on soil microbial communities. Additionally, soil chemical properties, cucumber root vigor, and fruit yield were measured to assess the impacts of vegetable residues incorporation on continuously cropped soil and cucumber growth.

Results: The results showed that incorporating vegetable residues could improve soil buffering capacity, increase the content of soil organic matter and available nutrients, and increased the diversity of soil microorganisms and improved community structure; vegetable residues increased the abundance of beneficial bacteria such as *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Chloroflexi*, while reducing the quantity of soil-borne pathogens such as *Bacillariophyta* and *Acidobacteria*. Similar results were observed for the fungal communities: the relative abundance of *Ascomycota* was decreased to varying degrees, while the relative abundance of *Rozellomycota* and *Basidiomycota* was raised. The results demonstrated that vegetable residues incorporation significantly increased cucumber root vigor and enhanced fruit yield. The effects of different types of residues on improving soil properties were ordered sweet pepper plant residues > cucumber plant residues > tomato plant residues, and 20% of sweet pepper plant residues incorporation had the most significant effect on crop yield.

[†]Xiaolu Chen and Dalong Zhang contributed equally to this work.

*Correspondence: minwei@sdau.edu.cn

[3] Shandong Collaborative Innovation Center for Fruit & Vegetable Production with High Quality and Efficiency, Tai'an, Shandong 271018, People's Republic of China

Full list of author information is available at the end of the article

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Conclusion: In summary, returning vegetable residues alleviated soil continuous cropping obstacles by improving the soil fertility and the diversity and community structure of soil microorganisms, and consequently promoting the growth and yield of greenhouse-grown cucumbers. The findings demonstrated that returning vegetable residues was an effective and sustainable measure for soil amendment during continuous cropping in greenhouse production.

Keywords: Vegetable residues, Continuously cropped soil, Soil microorganisms, Cucumber, Solar greenhouse

Introduction

Continuous cropping is commonly employed in intensive greenhouse production, but long-term continuous cropping may result in soil deterioration, inhibition of plant growth, and reduced yield and quality (Mendes et al. 2018; Liu et al. 2014). This well-known phenomenon, described as "soil sickness" or "continuous cropping obstacles", constrains the sustainable development of greenhouse production. Various factors contribute to soil sickness, such as accumulation of soil-borne pathogens and autotoxins, and deterioration of soil physical and chemical properties (Huang et al. 2013). Microbial community structure is the most active part of the soil microecological environment, and this has a significant influence on soil quality and plant growth (Mohanram and Kumar 2019; Chaparro et al. 2012). Continuous cropping reduces microbial diversity and soil organic matter (Lyu et al. 2020). Application of organic materials or microbial agents in agricultural practices can alleviate continuous cropping obstacles by manipulating the microbiome and increasing soil organic carbon (Ding et al. 2016; Wang et al. 2021). Crop residues retention can promote the growth and development of vegetables in continuous cropping soils by improving the physical and chemical properties, and increasing soil microbial diversity (Sun et al. 2011; Zhang et al. 2018a, b). However, most relevant reports focus on field crops such as maize, wheat, and rice straw (Qi et al. 2007; Zhang et al. 2013), and utilization of vegetable residues has received less attention than field crops.

With the rapid increase in vegetable cultivation area worldwide, large amounts of residues are produced during vegetable production (Pavi et al. 2017), most of which is discarded or buried in landfill, which negatively impacts both urban and rural ecology (Lipinski et al. 2013; Di Donato et al. 2011). In northern China, the most common vegetable species grown in solar greenhouses include tomatoes, sweet peppers, cucumbers etc. Effective utilization of vegetable residues is essential for sustainable vegetable production (Singh et al. 2012), especially greenhouse cultivation. So, we hope to find a reasonable disposal method to realize the reuse of them. Also from the perspective of providing nutrients for the soil, the decomposed vegetable residues are rich in nitrogen and carbon, which can provide nutrients for the soil and reduce the use of chemical fertilizers. The suitable types and concentrations of vegetable residues incorporation and the implications of alleviating soil continuous cropping obstacles in greenhouse vegetable production have not been thoroughly explored. In the present study, we investigated incorporation of three vegetable residues from cucumber, tomato, and sweet pepper in the soil during continuous cropping of cucumber in a greenhouse. In terms of alleviating soil continuous cropping obstacles, we examined the effect of vegetable residues retention on (1) soil microbiome community structure and diversity, and (2) root development, plant height, stem diameter, and yield for continuous cropping of cucumber in a solar greenhouse. We hypothesized that vegetable residues incorporation could affect the diversity and composition of rhizosphere microbial communities, increase the soil nutrients, and promote *Cucumis sativus L.* growth. The findings provided new insight into the effective utilization of vegetable residues and soil amendments for continuous cropping in greenhouse production.

Materials and methods

Vegetable residues preparation and soil sampling during continuous cropping

Experimental soil was collected from Daotian Town, Shouguang County, Shandong Province, China (118°96′E, 36°85′N). This site is a typical greenhouse vegetable production area, well known as the "vegetable village" of China. Tomatoes and cucumbers are the most widely grown vegetable crops, with plastic tunnels and solar greenhouses as the leading cultivation practices. A solar greenhouse with 18 years of continuous cucumber monoculture was selected for experimental soil collection. After being collected from the greenhouse, soil was stored in the shade for subsequent pot experiments.

Cucumber plant residues (CPR), tomato plant residues (TPR) and sweet pepper plant residues (SPR) were examined (on a dry basis; Table 1). Vegetable residues were carefully separated into above-ground and below-ground parts, and above-ground residues were chopped into tiny pieces (4-10 mm in length) using a pulverizer. Chopped vegetable residues were mixed with the special decomposition agent for residues (100 mg/kg), which was purchased from Henan Wobao Biotechnology Co., Ltd. The decomposition agent is rich in Bacillus, Trichoderma,

Table 1 Basic characteristics of examined vegetable residues

Treatments	Total carbon (mg/g)	Total nitrogen (mg/g)	C/N
CPR	526.00	34.57	15.22
TPR	444.82	19.88	22.38
SPR	498.87	17.91	27.85

and Saccharomyces. First, soak the crushed residues with water, and then sprinkle a layer of decomposition agent each layer of vegetable residues about 20 cm. Piled up the mixture with 50–60% water content, covered with plastic film, and the temperature could reach 60 °C within 3 days of decomposing. After about 20 days, the vegetable residues turned a uniform brown, indicating that the decomposition was completed. The prepared substrate was turned loose and waited for the experiment.

Experimental design

The vegetable residues incorporation experiment was conducted from August 2020 to February 2021 in a solar greenhouse of the Horticultural Experiment Station of Shandong Agricultural University (117°16′E, 36°17′N). Experiments were performed using a complete randomized block design with 10 replicates per treatment. In addition to control soil without residues incorporation (the soil with 18 years of continuous cucumber monoculture), nine treatments were established with residues incorporation; crushed CPR, TPR, and SPR were incorporated into air-dried soil at levels of 10%, 20%, and 30% (the volume calculation for soil and plant residues, v/v). Each treatment was divided into three plots, with 10 pots in each plot. Pots were uniformly filled with 10 kg of control soil without residues or well-mixed soil with residues. One healthy cucumber plant (cv. 'Changqing No. 1'/'Baimutian' grafted seedlings, Shandong Anxin Seedling Co., Ltd.) was transplanted into each pot, with a pot spacing of 40 cm and a row spacing of 25 cm. Plants were uniformly watered by drip irrigation. Routine management was carried out during cucumber growth.

Determination of plant growth and physiological parameters

Height and stem thicknesses of cucumber plants were measured 60 days after planting, by tape measure and vernier calipers, respectively. From the beginning to the end of the cucumber set, the yield of each plot was measured cumulatively using an electronic balance to determine the fresh mass of fruits. And cucumber root vigor was measured 60 days after planting using the triphenyl tetrazolium chloride (TTC) reduction method as described previously (Zheng et al. 2016). At first, the 0.5 g apical roots were weighed and put into graduated test tubes, followed by addition of 5 mL 0.4% TTC solution and 5 mL phosphate buffer (0.066 M, pH = 7.0). Later, the roots were fully immersed in the liquid, maintained for 2 h at 37 °C, and then, 2 mL of 1 M H_2SO_4 was added to terminate the reaction. Afterwards, the roots were taken out and wiped dry, adding 4 mL ethyl acetate and a little quartz sand in the mortar and grinded into homogenate. Then, the red leach liquor was poured into 10 mL tube. The optical density (OD) values were recorded with a UV-Vis recording spectrophotometer at 485 nm. The TTC concentration was obtained from the standard curve. The strength of root reductive TTC (root vigor) was determined as follows:

$$\text{Root vigor}\left(\text{mg/kg/h FW}\right) = \text{The reducing amount of TTC}\left(\text{mg}\right)/\text{Root weight}\left(\text{kg}\right)\times\text{Time (h)}$$

Collection of soil sample following residues retention

Substrate samples of mixed soil and residues were collected before cultivation and after cucumber plant harvest. Surface soil was removed and rhizosphere soil was collected at a depth of 0-20 cm, about 5 cm from the roots. Each sample was further divided into two subsamples. One subsample was used for chemical property analysis after drying in air and passing through a 2-mm mesh, and the other was stored at − 80 °C until microbial community analysis after removing impurities.

Soil chemical properties

Soil chemical properties were measured as described previously according to the methods of Bao (2000). (1) Soil pH was determined according to the electrode method at a 1:2.5 ratio of soil-to-water (m:v). (2) Soil organic matter (SOM) content was assayed with acidified potassium dichromate ($K_2Cr_2O_7$–H_2SO_4) by the titration method. (3) Soil total nitrogen (TN) content was measured according to the Kjeldahl method. (4) Soil available nitrogen (AN) content was determined by alkaline hydrolysis diffusion method. (5) Soil total phosphorus (TP) was extracted by means of microwave digestion in sulfuric acid and H_2O_2 and determined by the ascorbic acidmolybdenum blue method. (6) Soil available phosphorus (AP) was extracted by 0.5 M $NaHCO_3$ and determined using the Olsen-P method by spectrophotometer (880 nm). (7) Soil total potassium (TK) was extracted

using the sodium hydroxide melting method. (8) Soil available potassium (AK) was extracted by 1 M NH_4OAc (pH = 7.0). Contents of TK and AK were determined photometrically by a flame spectrophotometer.

DNA extraction and PCR amplification

Microbial community genomic DNA was extracted from samples using an E.Z.N.A. Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, Wilmington, USA).

The V3—V4 hypervariable region of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) using an ABI GeneAmp 9700 PCR thermocycler (ABI, CA, USA). PCR amplification of the 16S rRNA gene was performed with an initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min, then storing at 4 °C.

The fungal ITS rRNA gene was amplified with primer pairs ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). The PCR amplification equipment and parameters of the ITS rRNA gene were carried out as described for bacterial.

PCR mixtures contained 5× TransStart FastPfu buffer (4 μL), 2.5 mM dNTPs (2 μL), 5 μM forward primer (0.8 μL), 5 μM reverse primer (0.8 μL), TransStart FastPfu DNA Polymerase (0.4 μL), template DNA (10 ng), and ddH_2O up to 20 μL. Amplifications were performed in triplicate, and PCR products were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to manufacturer's instructions and quantified using a Quantus Fluorimeter (Promega, Madison, WI, USA).

Illumina MiSeq sequencing

Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 300) on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) according to standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

Statistics and analysis

Soil microbial high-throughput sequencing data analysis was performed based on the cloud service (https://www.i-sanger.com) provided by Major Bio-Pharm Technology Co. Ltd. (Shanghai, China). For detailed data analysis software and algorithms, refer to the official Majorbio Bio-Pharm Technology Co. Ltd. website (URL). Before analysis, data were arranged according to the minimum number of sample sequences. Principal coordinate analysis (PCoA) based on Bray-Curtis similarity distances and weighting of UniFrac distances were carried out in the R package vegan (v 3.3.1). In order to link bacterial community structure to possible soil functions, PICRUSt software (http://picrust.github.io/picrust/) was used to make functional predictions for observed bacterial sequences. The relative abundance of the second-level Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways was compared among sample types.

Microsoft Excel 2016 was used for data processing and graphing. Soil properties data were statistically analyzed using SPSS 21.0 software and Duncan's new compound polar difference method was used to assess the significance of differences ($p < 0.05$).

Results

Soil chemical properties analysis

Before planting cucumbers, soil organic matter and available nutrients increased with the increase of the quantity of vegetable residues application, because of the high content of carbon and other nutrients in vegetable residues. The effect of SPR and CPR incorporation to improve soil pH value was better than that of TPR at 60 days after planting (DAP). With the growth of cucumber, soil organic matter and other nutrients were absorbed and decomposed, but they declined to different degrees in different treatments. For example, CPR were beneficial to increase soil organic matter and available phosphorus content, and SPR were beneficial to increase available potassium content (Table 2).

Quality analysis of sequencing results

High-throughput sequencing yielded 1,181,776 valid bacterial sequences and 1,292,604 valid fungal sequences from 20 samples (Table 3). Based on 97% sequence similarity, the length of bacterial library sequences ranged from 206 to 531 bp, and 5531 operational taxonomic units (OTUs) were obtained. The length of fungal library sequences ranged from 140 to 538 bp, and 924 OTUs were acquired based on 97% similarity.

Rarefaction curve analysis revealed the sampling depth for each sample, which reflected the adequacy of the sample library sequencing data volume, and this could be used to evaluate whether sequencing data can cover all taxa. Bacterial and fungal diversity rarefaction curves (Fig. 1) were flattened (i.e., they reached saturation) when the sequencing volume exceeded 40,000 read lengths, indicating that the amount of sequencing data was adequate and could reflect most sequence information for soil bacterial and fungal communities.

Table 2 Soil chemical properties analysis of vegetable residues application and continuous cropping soils

Periods	Treatments	pH	SOM (g/kg)	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)
Before planting	ACK	7.48 ± 0.03d	29.29 ± 2.22d	188.07 ± 2.85e	127.47 ± 1.65f	131.63 ± 0.00g
	AC10	7.55 ± 0.06bcd	36.00 ± 2.78bcd	196.70 ± 4.96e	139.21 ± 2.58cd	134.12 ± 2.49g
	AC20	7.68 ± 0.06ab	38.87 ± 1.20abc	218.17 ± 2.91d	149.91 ± 2.67b	151.56 ± 2.49d
	AC30	7.69 ± 0.10ab	43.79 ± 2.45a	251.18 ± 457a	180.10 ± 7.96a	187.27 ± 1.44a
	AP10	7.64 ± 0.07bc	34.97 ± 1.72bcd	212.45 ± 7.48d	131.17 ± 4.75ef	144.08 ± 0.83e
	AP20	7.70 ± 0.06ab	37.66 ± 2.56abc	234.62 ± 6.88c	137.53 ± 2.12de	170.66 ± 0.83c
	AP30	7.79 ± 0.12a	41.73 ± 3.16ab	241.27 ± 7.78bc	145.22 ± 1.65bc	189.76 ± 2.19a
	AT10	7.52 ± 0.10cd	31.81 ± 2.65cd	244.77 ± 3.71ab	132.95 ± 1.02ef	138.27 ± 1.44f
	AT20	7.58 ± 0.08bcd	35.88 ± 1.22bcd	245.58 ± 3.64ab	138.69 ± 3.97d	144.08 ± 3.80e
	AT30	7.58 ± 0.04bcd	40.41 ± 3.27ab	249.20 ± 3.55ab	148.58 ± 0.96b	183.94 ± 1.43b
60 DAP	BCK	7.53 ± 0.03c	23.55 ± 0.77c	204.16 ± 8.93d	133.54 ± 1.12d	120.73 ± 4.29f
	BC10	7.60 ± 0.03bc	26.94 ± 1.05abc	212.8 ± 9.20d	147.42 ± 2.78cd	128.21 ± 1.63e
	BC20	7.87 ± 0.02a	28.89 ± 1.43abc	219.8 ± 9.05bc	154.13 ± 3.42c	140.38 ± 1.62d
	BC30	7.89 ± 0.05a	30.73 ± 2.13a	254.1 ± 3.10a	188.48 ± 7.29a	245.18 ± 0.00b
	BP10	7.66 ± 0.10b	27.45 ± 2.21abc	219.45 ± 10.60bc	144.18 ± 3.52cd	138.51 ± 0.00d
	BP20	7.78 ± 0.06a	28.72 ± 0.71abc	232.98 ± 4.15b	148.81 ± 5.51cd	221.79 ± 9.86c
	BP30	7.82 ± 0.11a	29.75 ± 1.49ab	251.3 ± 4.96a	154.48 ± 9.75c	260.15 ± 3.24a
	BT10	7.60 ± 0.04bc	24.94 ± 0.96bc	223.42 ± 1.46bc	152.97 ± 9.29c	136.64 ± 1.62d
	BT20	7.62 ± 0.01bc	27.63 ± 0.61abc	229.95 ± 1.60b	149.97 ± 3.28cd	142.25 ± 1.62d
	BT30	7.65 ± 0.05bc	27.92 ± 0.57abc	250.25 ± 5.97a	171.48 ± 2.95b	222.72 ± 2.81c

CK Continuous cropping soil without residues application, *C10* 10% application of CPR, *C20* 20% CPR, *C30* 30% CPR, *P10* 10% SPR, *P20* 20% SPR, *P30* 30% SPR, *T10* 10% TPR, *T20* 20% TPR, *T30* 30% TPR. In front of each treatment abbreviation, A represents before planting and B represents 60 days after planting. Values are means ± standard deviation ($n = 3$). Different lower case letters refer to significant differences between groups based on Duncan's new compound polar difference method ($p < 0.05$). The same groups apply below

Microbial diversity analysis

In order to assess the rhizosphere microbial diversity subjected to different treatments, alpha diversity indices were studied. Incorporation of all three vegetable residues significantly increased the diversity of the soil bacterial communities, compared with control soil without vegetable residues (Table 4). The bacterial diversity following incorporation of SPR before planting was higher than that of other treatments. Overall, the soil bacterial diversity and richness index were higher for CPR application than for other treatments at 60 DAP, and the 20% application rate was highest. The effect on the diversity of fungal communities was significantly higher than the effect on bacterial communities. Under the same application rate of vegetable residues, the highest fungal diversity was observed for the CPR incorporation both before and after planting.

Ace and Chao indices can reflect the species richness of a community. As can be seen in Table 4, vegetable residues could also increase soil bacterial community richness. The change in bacterial richness was consistent with the trend in diversity, and the highest richness was observed for the 20% application of CPR at 60 DAP.

The effect of vegetable residues on fungal community abundance before and after planting varied considerably. The fungal richness of TPR returned to the field before planting was lower than that of controls. The fungal richness with CPR application was higher than that of other treatments, all treatments were significantly higher than controls at 60 DAP, and the highest fungal richness was observed for 20% of CPR application both before and after planting.

Microbial community composition dissimilarity analysis

Principal coordinate analysis (PCoA), a standard method for analyzing beta-diversity, is a non-constrained method for dimensionality reduction of data that can be used to study similarities or differences in the composition of sample communities. In this study, PCoA was performed based on the Bray-Curtis similarity distances (bacterial Fig. 2A, fungal Fig. 2B) and weighting of UniFrac distances (bacterial Fig. 2C, fungal Fig. 2D). Differences between individuals or groups can be assessed by the length of sample points on graphs; the closer the sample point, the more similar the microbial community composition.

Table 3 Sample data statistics of vegetable residues application and continuous cropping soils

Periods	Treatments	Bacteria 338F_806R		Fungi ITS1F_ITS2R	
		Sequence reads	Average length	Sequence reads	Average length
Before planting	ACK	48,817	418.74	69,158	258.34
	AC10	47,207	416.47	67,330	236.17
	AC20	53,998	417.97	70,051	241.12
	AC30	62,635	417.97	60,433	246.94
	AP10	56,633	417.24	73,500	252.30
	AP20	67,459	418.91	56,503	249.04
	AP30	74,037	418.64	62,852	249.45
	AT10	57,395	418.38	70,441	252.86
	AT20	59,250	419.56	66,634	258.75
	AT30	60,853	417.87	66,972	253.78
60 DAP	BCK	63,117	416.52	57,497	252.91
	BC10	61,079	416.41	57,210	246.15
	BC20	59,204	416.39	74,174	231.22
	BC30	66,365	417.12	64,200	249.88
	BP10	62,395	417.99	67,735	238.49
	BP20	52,150	417.05	66,353	251.61
	BP30	52,053	416.52	61,503	248.75
	BT10	54,083	417.24	53,557	270.38
	BT20	57,350	416.83	61,367	250.93
	BT30	65,696	417.29	65,134	245.16

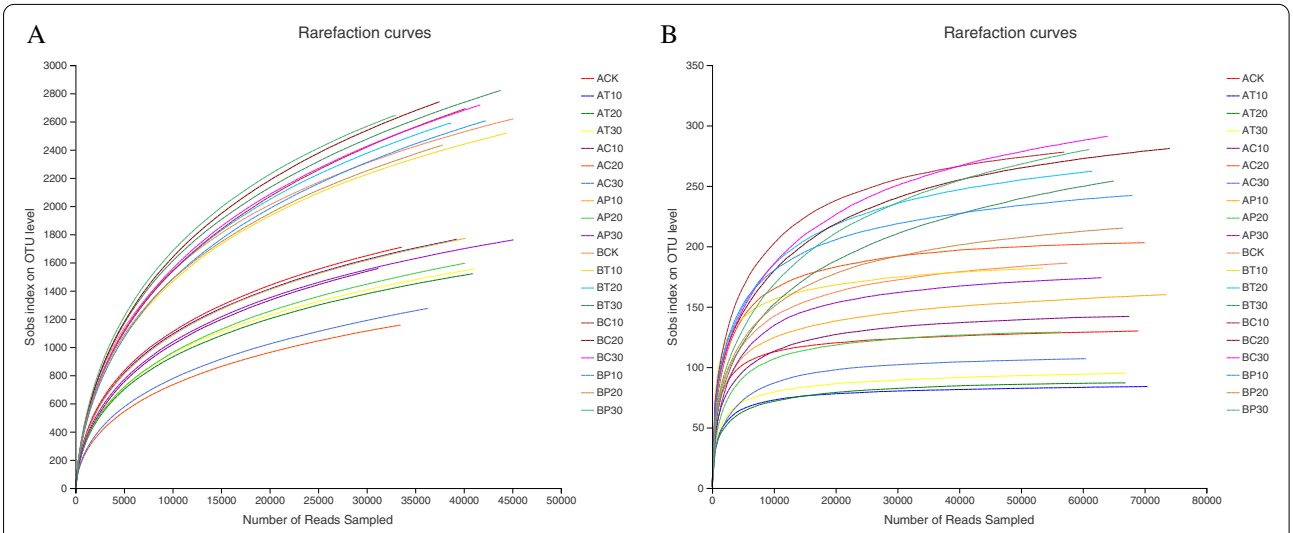

Fig. 1 Rarefaction curves of different vegetable residues application. **A** Rarefaction curves of bacterial communities. **B** Rarefaction curves of fungal communities

The contribution of the 1st and 2nd principal components of the bacterial communities based on PCoA weighted using Bray-Curtis and UniFrac distances were 28.15%, 23.53%, 33.69%, and 25.09%, respectively (Fig. 2). The contribution of the 1st and 2nd principal components of PCoA on the fungal communities was 67.33%, 13.92%, 82.42%, and 8.43%, respectively. These results indicated that all principal coordinates selected in PcoA

Table 4 Diversity analysis of soil microbial communities in different vegetable residues application

Microorganisms	Periods	Treatments	Community diversity	Community richness			Community coverage
			Shannon index	Simpson index	Ace index	Chao index	Coverage (%)
Bacteria	Before planting	ACK	4.70	0.021	2092.83	2054.41	0.984
		AC10	5.37	0.014	2180.76	2240.76	0.983
		AC20	5.27	0.017	2222.48	2374.76	0.988
		AC30	4.99	0.019	2110.59	2355.43	0.988
		AP10	5.45	0.012	2223.11	2158.21	0.987
		AP20	5.51	0.011	2394.96	2179.70	0.987
		AP30	5.56	0.010	2294.19	2309.01	0.988
		AT10	5.37	0.012	2163.93	2169.35	0.986
		AT20	5.38	0.015	2369.82	2301.65	0.988
		AT30	5.52	0.011	2302.90	2246.02	0.988
	60 DAP	BCK	6.11	0.008	3217.47	3245.69	0.983
		BC10	6.16	0.008	3929.13	3777.23	0.976
		BC20	6.21	0.010	3778.52	3876.72	0.975
		BC30	6.13	0.008	3715.31	3794.42	0.978
		BP10	6.09	0.009	3638.51	3356.65	0.979
		BP20	6.19	0.009	3307.30	3614.88	0.979
		BP30	6.14	0.005	3563.68	3550.09	0.974
		BT10	6.17	0.007	3375.55	3480.12	0.983
		BT20	6.14	0.010	3517.53	3537.75	0.978
		BT30	6.26	0.006	3779.56	3718.39	0.978
Fungi	Before planting	ACK	1.84	0.107	134.90	139.33	0.9999
		AC10	2.66	0.144	144.93	144.10	0.9999
		AC20	2.74	0.143	205.92	204.62	0.9999
		AC30	2.11	0.196	108.64	108.43	0.9999
		AP10	2.72	0.155	171.06	170.50	0.9998
		AP20	2.04	0.291	181.00	180.00	0.9999
		AP30	2.06	0.264	131.32	137.13	0.9998
		AT10	2.39	0.169	87.18	85.50	0.9999
		AT20	2.47	0.127	88.36	87.43	1.0000
		AT30	1.94	0.406	99.49	100.25	0.9999
	60 DAP	CCK	3.06	0.092	196.55	198.21	0.9997
		BC10	3.52	0.065	294.32	294.73	0.9995
		BC20	3.75	0.221	331.14	336.62	0.9995
		BC30	3.54	0.057	318.94	306.54	0.9992
		BP10	3.10	0.093	258.76	257.79	0.9996
		BP20	3.16	0.070	228.43	232.65	0.9996
		BP30	3.06	0.088	327.41	339.03	0.9990
		BT10	3.37	0.066	257.55	258.88	0.9998
		BT20	3.66	0.068	281.40	284.96	0.9995
		BT30	2.54	0.172	296.97	311.24	0.9992

could adequately explain the differences in the original data. The range of changes in the fungal communities was much more extensive than that of the bacterial communities before and after planting. At 60 DAP, samples treated with vegetable residues were distributed on the positive axis of principal component 1. By contrast, the control group (BCK) was spread on the negative axis of central component 1 (Fig. 2B, D). The results showed that

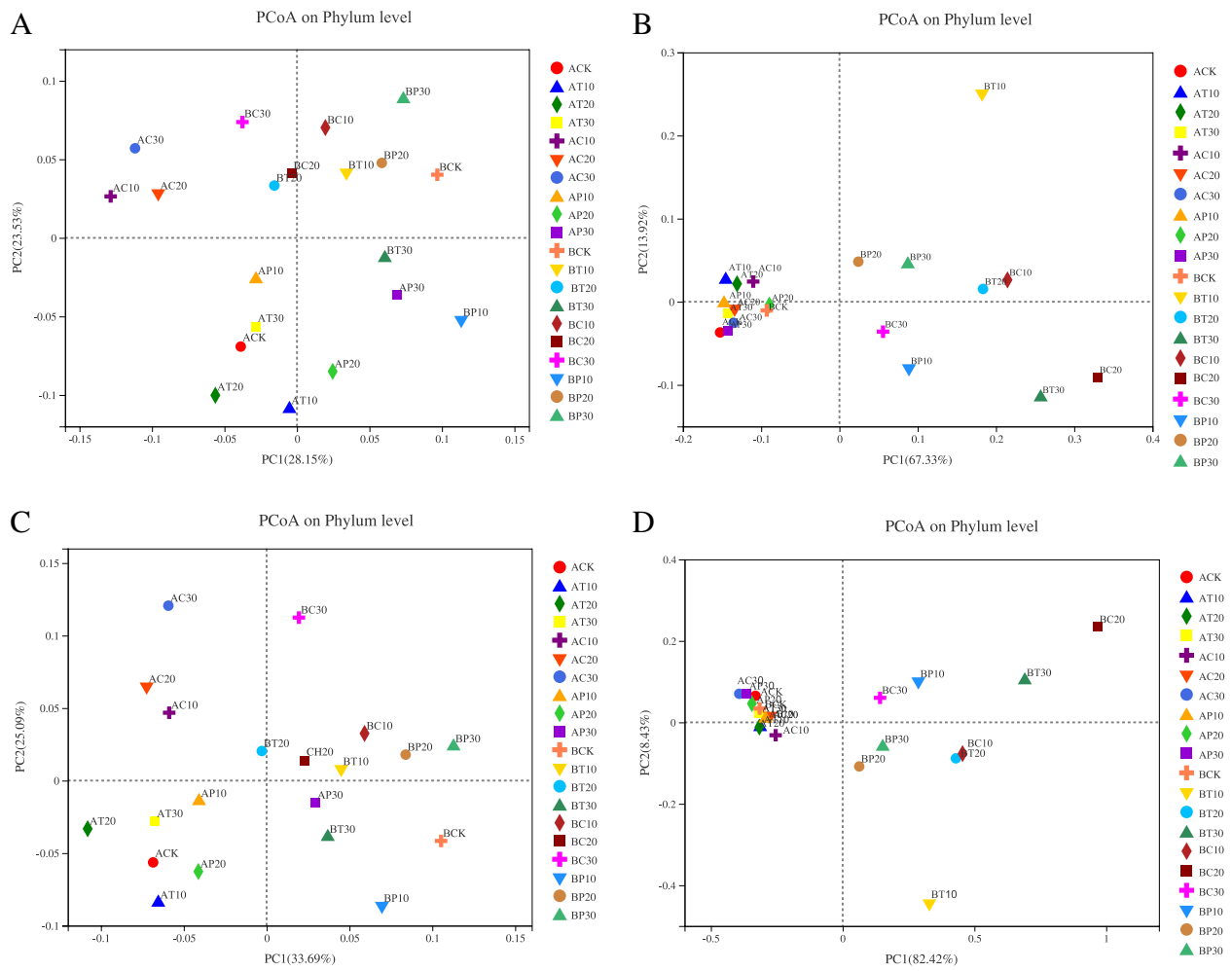

Fig. 2 Principal coordinate analysis of bacterial and fungal communities based on Bray-Curtis similarity distances and weighting of UniFrac distances. **A** PCoA plots of bacterial communities based on Bray-Curtis similarity distances. **B** PCoA plots of fungal communities based on Bray-Curtis similarity distances. **C** PCoA plots of bacterial communities based on weighting of UniFrac distances. D PCoA plots of fungal communities based on weighting of UniFrac distances. PCoA plots based on Bray-Curtis and weighted-UniFrac distances

the community structure of soil fungi in each treatment group was significantly different from that in the control group.

Microbial community composition analysis

The relative abundance and composition of microorganisms were similar among different treatments. A remarkable difference was observed in the relative abundance of the dominant phylum among treatments (Figs. 3 and 4).

The community distribution and relative abundance at the bacterial phylum level are shown in Fig. 3. Bacteria in soil samples included *Actinobacteriota*, *Firmicutes*, *Proteobacteria*, *Chloroflexi*, *Gemmatimonadota*, *Bacteroidota*, and *Acidobacteriota*. In addition to the unclassified groups, *Actinobacteriota*, *Firmicutes*, *Proteobacteria*, and *Chloroflexi* were the dominant bacteria with relatively high abundances.

Vegetable residues incorporation increased the relative abundance of *Actinobacteriota*, *Firmicutes*, *Proteobacteria*, and *Chloroflexi*, and decreased the relative abundance of *Gemmatimonadota* and *Acidobacteriota*. Compared with the control group (BCK), the percentage of *Actinobacteriota* increased by 36.35%, 58.38%, 43.67%, 45.23%, 51.61%, 41.62%, 5.77%, 9.77%, and 3% in CPR, SPR, and TPR treatments, respectively. The percentage of *Firmicutes* increased by 14.2%, 28.19%, 57.68%, 5.17%, 27.02%, 13.11%, 73.05%, 38.46%, and 25.35%. Meanwhile, the percentage of *Gemmatimonadota* decreased by 29.84%, 41.59%, 49.81%, 35.78%, 46.14%, 53.1%, 32.49%,

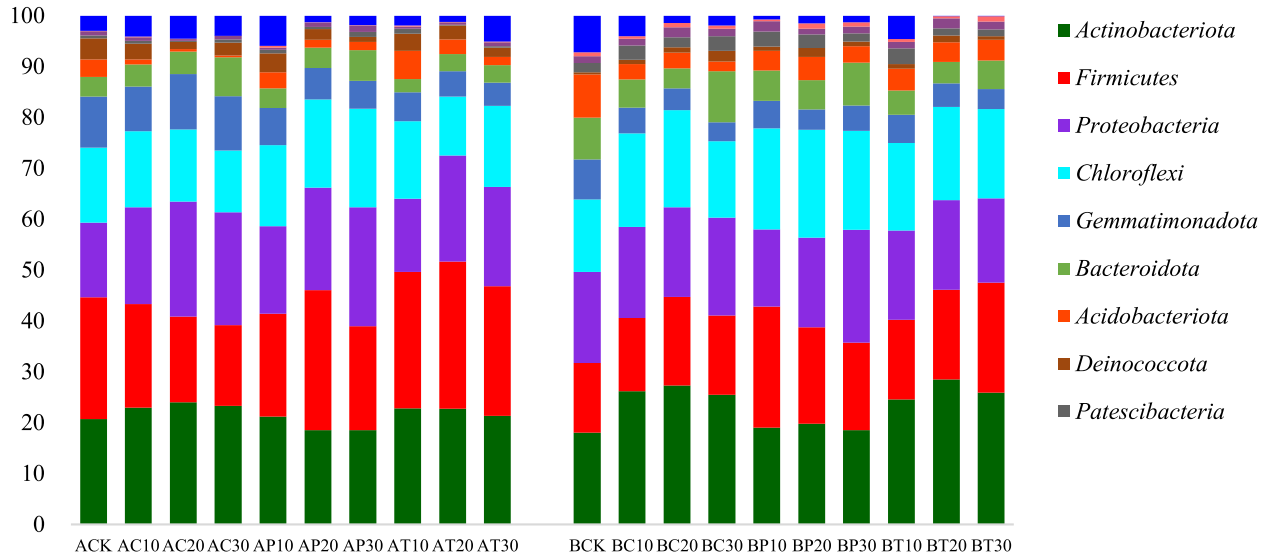

Fig. 3 Composition and relative abundance of the main bacterial communities in different treatments

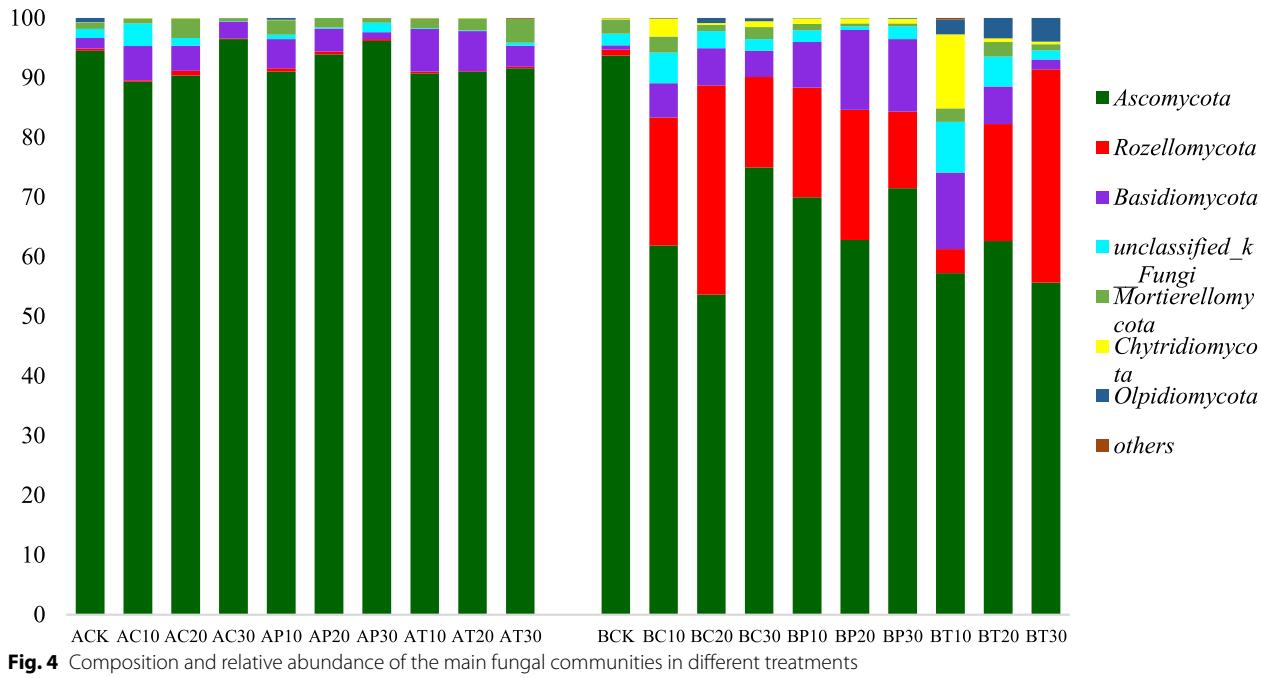

Fig. 4 Composition and relative abundance of the main fungal communities in different treatments

49.68%, and 36.92%, respectively. Compared with the control group, *Acidobacteriota* was decreased by 49.94%, 54.66%, 52.42%, 64.46%, 63.64%, 78.04%, 54.66%, 46.16%, and 62.22%, respectively.

The distribution and relative abundance of communities at the fungal phylum level are shown in Fig. 4. The 4th ranked group in terms of relative abundance was not classified due to database limitations. *Ascomycota*, *Rozellomycota*, and *Basidiomycota* were the most abundant fungal phyla in soil samples for all treatments, with *Ascomycota* the dominant fungal group, accounting for 55.66-96.47% of the relative abundance.

Figure 4 shows that returning vegetable residues to the field could reduce the relative abundance of *Ascomycota* and increase the relative abundance of *Rozellomycota* and *Basidiomycota* to varying degrees. The relative

abundance of the *Ascomycota* phylum was reduced by 38.98%, 33.19%, 40.64%, 34.02%, 42.77%, 20.02%, 25.39%, 33.02%, and 23.72%, respectively, at 60 DAP compared to controls. The relative abundance of *Rozellomycota* and *Basidiomycota* varied greatly, with the relative abundance of *Rozellomycota* increasing 3.2-35.9 times and *Basidiomycota* increasing 1.2-16.4 times compared to controls at 60 DAP.

Metabolic functions analysis of the bacterial communities

The metabolic functions of the bacterial communities were predicted using PICRUSt software based on the KEGG database at the second classification level. Figure 5 showed the KEGG gene metabolic pathway entries in the top 17 of relative abundance. According to their functions, they can be classified into four categories: metabolism, genetic information processing, environmental information processing, and cellular processes. The relative abundance of some metabolic pathways closely related to soil material cycle in SPR treatments were higher than these in CPR and TPR treatments. For example, the relative abundance of amino acid metabolism and membrane transport in 20% BPR was significantly higher than that in other treatments, which was 5.83% and 5.19% higher than that of CK at 60 DAP, respectively. The same results can be seen in other metabolic pathways (i.e., lipid metabolism, replication and repair).

Effect of different vegetable residues on cucumber growth

Vegetable residues incorporation had no significant effect on plant height (Fig. 6A). Plant height increased with increasing levels of identical vegetable residues, but no significant differences were observed among treatments. TPR had the most potent effect on plant height and stem diameter for all amounts of different types of residues. Returning vegetable residues to the field could promote the enlargement of cucumber stem diameter; the largest cucumber stem diameter was achieved with 20% of CPR application, an increase of 16.1% compared with controls.

Effect of different vegetable residues on cucumber root vigor

In addition to the 30% TPR treatment, the root vigor of cucumber in continuous cropping soil could be significantly increased by returning other vegetable residues to the field (Fig. 7). The root vigor of cucumber in the P20 treatment reached 33.78 mg/kg/h FW, 74.8% higher than that of CK. Root vigor was increased most by the 20% treatments for all three kinds of residues.

Effect of different vegetable residues on cucumber yield

Cucumber yield increased with increasing vegetable residues application and peaked at 20% addition for all three vegetable residues types (Fig. 8). Specifically, 20%

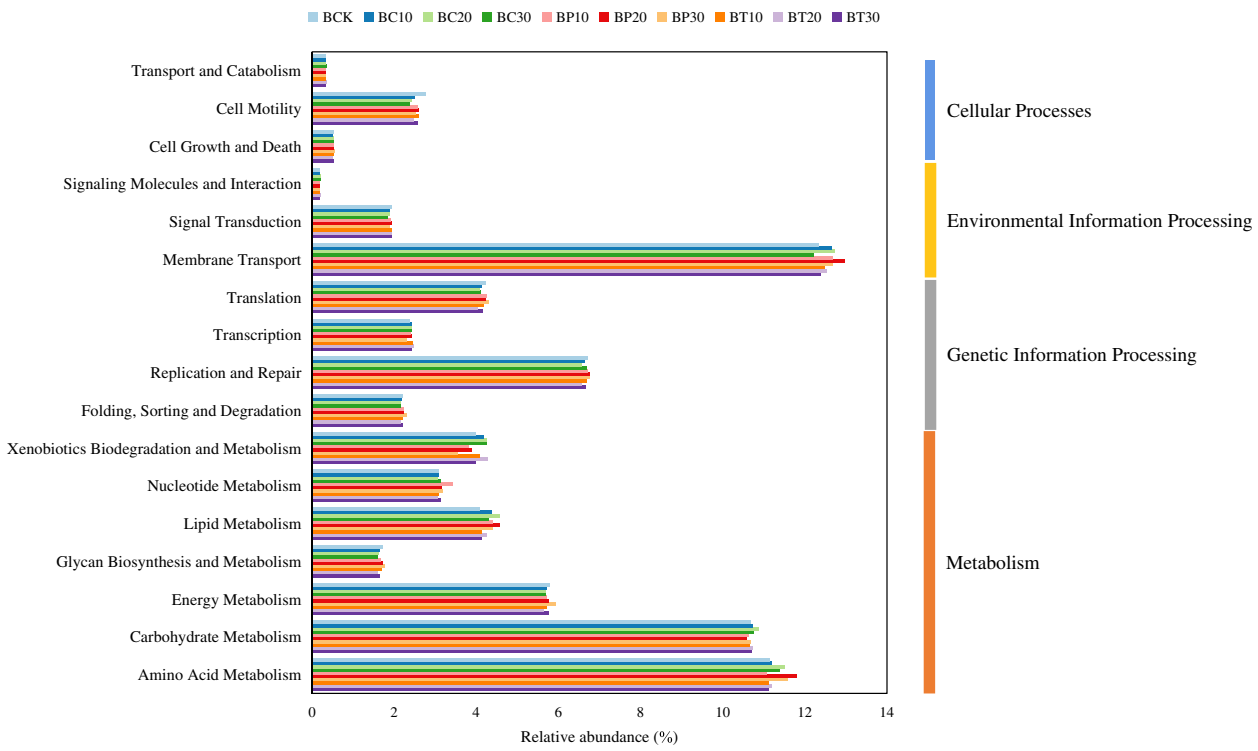

Fig. 5 The abundance of the metabolic functions of the bacterial communities at the second classification level

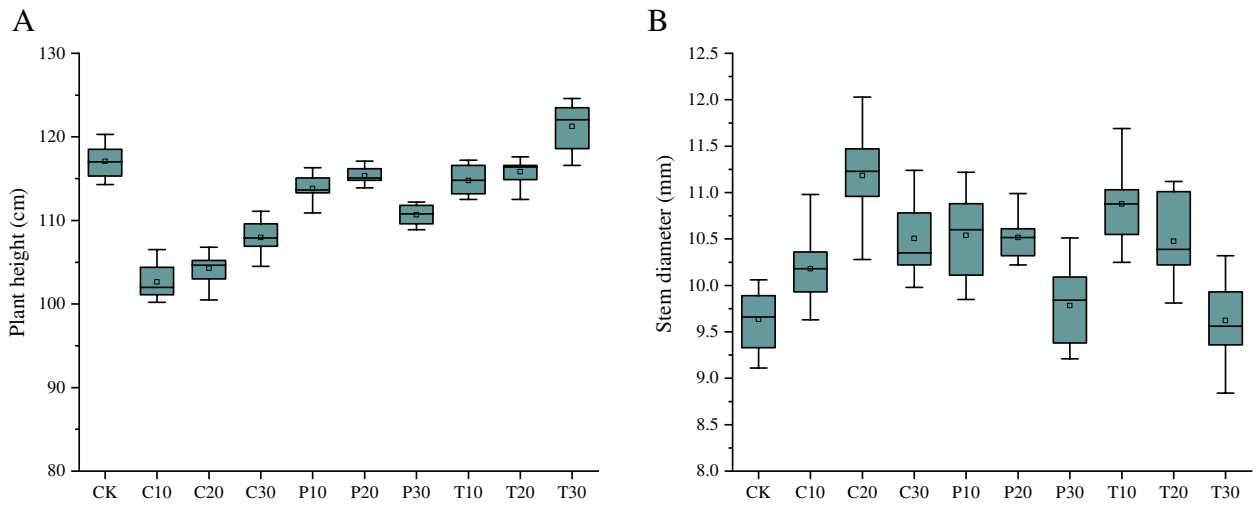

Fig. 6 Effect of different vegetable residues on the plant height and stem diameter of cucumber after 60 DAP

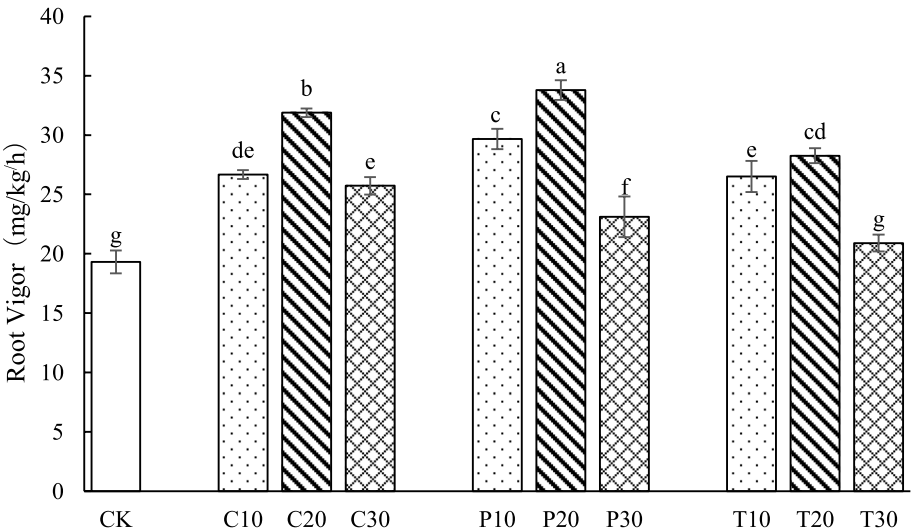

Fig. 7 Effect of different vegetable residues on the root vigor of cucumber after 60 DAP

addition of SPR, TPR, or CPR increased fruit yield by 11.9%, 7.7%, and 7.2%, respectively, compared with control soil. Cucumber yield was highest following the addition of SPR, regardless of the quantity of residues added.

Discussion

In this study, we demonstrated that incorporating vegetable residues could moderate soil deterioration in continuous cucumber monoculture. All three types of vegetable residues improved microbial diversity and community structure, and the relative abundance of beneficial bacteria was increased. The improved soil microbiomes enhanced the growth and yield of greenhouse-grown cucumber, suggesting that returning vegetable residues to soil was an effective and sustainable soil amendment measure for continuous cropping in greenhouse production.

Incorporating vegetable residues can increase soil quality and fertility

As a carbon-rich biomass, crop residues contain carbon (40–45%), nitrogen (0.6–1%), phosphorus (0.45–2%), potassium (14–23%), and microelements, which are necessary for crop growth (Wang et al. 2020). In this study, we found that all three kinds of vegetable residues could

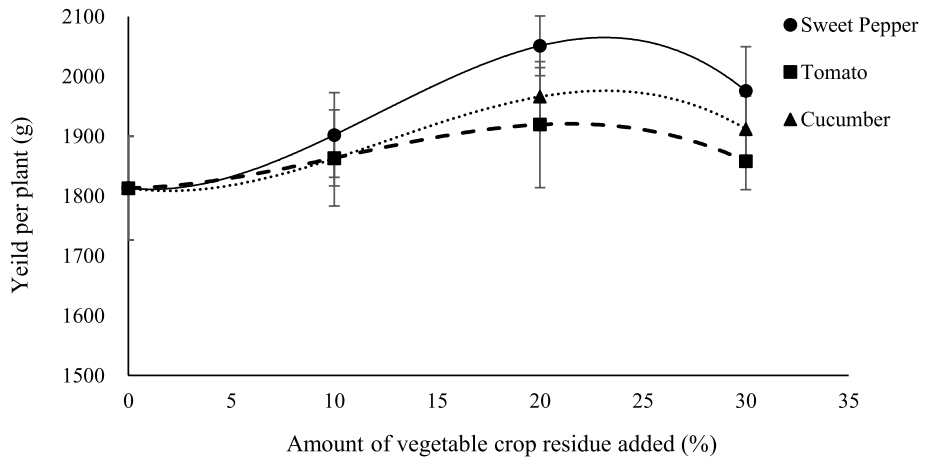

Fig. 8 Effect of vegetable residues on cucumber yield for different amounts added

trim the soil pH value, improve soil buffering capacity and increase the content of soil organic matter and available nutrients. Because of the different properties of vegetable residues (C/N ratio and chemical composition), the effect of soil improvement is also different. Combined with the results in Tables 1 and 2, the content of soil organic matter with CPR incorporation was higher than that of other treatments because of the high content of carbon in CPR. The same results have been found in other studies (Zhu et al. 2014; Zhang et al. 2018a, b).

Incorporating vegetable residues moderates soil deterioration by improving soil microbial diversity

Soil microorganisms play a crucial role in many biochemical processes that are essential for the environmental, ecological and production functions of soils, hence they are useful quality indicators (Mierzwa-Hersztek et al. 2019). Numerous studies have shown that returning crop residues to the field was a powerful way to effectively utilize residues to increase soil nutrient content and adjust soil microbial community structure and diversity (Ilieva-Makulec et al. 2006; Maluf et al. 2015). In the present study, Shannon and Simpson indices were used to reflect the diversity of microorganisms in soil samples; the higher the Shannon index, the higher the community diversity, and the higher the Simpson index, the lower the community diversity. The ACE index is used to estimate the number of OTUs in the community, and the Chao index is commonly used in ecology to estimate the total number of species. Herein, the diversity and richness of soil microbial communities could be enhanced to varying degrees by returning different vegetable residues to the field. This could be because vegetable residues are rich in organic matter and other kinds of nutrients, which can provide abundant carbon and nutrients for soil microbial activities, thereby increasing the activity of soil microorganisms (Schipanski et al. 2014; Snapp et al. 2005). Also, microbial diversity/richness increase at 60 DAP in comparison with before planting. On the one hand, cucumber roots produce root exudates which have been shown to play an important role in root-microorganisms interactions (Walker et al. 2003). On the other hand, as a consequence of normal growth and development, a large range of organic and inorganic substances are secreted by roots into the soil, which inevitably leads to changes in soil physical and chemical properties (Rougier 1981). This also has an indirect effect on soil microorganisms.

Incorporating vegetable residues moderates soil deterioration by improving soil microbial community structure

High-throughput sequencing was used to investigate the effects of vegetable residues incorporation on soil microbial community structure. The microbial community composition following incorporation of different vegetable residues was similar, but there were some differences. This indicated that vegetable residues affected the microbial community composition of the soil.

We analyzed the distribution characteristics of dominant bacteria in the ground under different treatments and found that the relative abundances of *Actinobacteriota*, *Firmicutes*, *Proteobacteria*, and *Chloroflexi* were higher than those of other bacterial phyla except some unclassified ones, consistent with the predominant bacterial phyla identified in other studies (Chen et al. 2018). The results also showed that the relative abundance of *Actinobacteriota*, *Firmicutes*, *Proteobacteria*, and *Chloroflexi* could be increased by vegetable residues incorporation, while the relative abundance of *Gemmatimonadota* and *Acidobacteriota* could be decreased (Fig. 3). Previous

studies have shown that *Actinobacteriota* can decompose cellulose and lignin, and abundant *Actinobacteriota* are beneficial for the decomposition of organic plant residues in soil (Kanokratana et al. 2011; Kausar et al. 2011). *Firmicutes* can increase the mass ratio of fast-acting phosphorus (Liu et al. 2018), and *Chloroflexi* can generate energy through photosynthesis, making them useful for degradation of soil pollutants (Bennett et al. 2020). The content of available nutrients in the rhizosphere soil of cucumber increased with increasing *Proteobacteria* and decreasing *Acidobacteriota* (Yang et al. 2021). With the rapid growth in the number of sequenced genomes, the PICRUSt tool has been increasingly used to infer functions that are likely associated with a marker gene based on its sequence similarity with a reference genome (Alami et al. 2020; Chen et al. 2020). In this study, the prediction of bacterial communities function found that the relative abundances of main metabolic pathways in various vegetable residues treatments were higher than CK.

The distribution characteristics of the dominant fungal communities in each treatment were analyzed, and the effects of vegetable residues returned to the field on changes in the fungal communities were more significant than for changes in the bacterial communities. Most notably, vegetable residues incorporation reduced the relative abundance of *Ascomycota* and increased the relative abundance of *Rozellomycota* and *Basidiomycota* to varying degrees (Fig. 4). This could improve the diversity and abundance of soil fungal communities (Table 4). *Ascomycota* members are mostly saprophytic and play an important role in the decomposition of plant residues and the degradation of soil organic matter (Bastida et al. 2016), while *Basidiomycota* species are important decomposers in soil because they breakdown cellulose, hemicellulose and lignin (Baldoni et al. 2014). Together, these results showed that vegetable residues could improve the content of beneficial microorganisms in soil and reduce the number of harmful or pathogenic microorganisms.

Vegetable residues improve plant growth and increase cucumber yields

The results showed that the effects of crop residues incorporation on crop yield differed between treatments. Returning crop residues to the field can improve soil available nutrients and soil quality, enhance soil water-holding and thermal insulation capacities, enrich soil microbial diversity, optimize the root growth environment, and promote root growth, thereby promoting crop productivity (Basak et al. 2012; Smitha et al. 2019). However, some studies showed that returning straw improved soil quality, but it did not affect crop yield, and even caused a yield reduction in some cases (Zhu et al. 2011; Bijay-Singh et al. 2008). Our current experiments showed that vegetable residues incorporation could significantly increase root vigor in cucumber. Although all treatments increased cucumber yield, most increases were not significantly different ($p > 0.05$). Only 20% of SPR and 20% of CPR incorporation substantially improved cucumber yield (Fig. 8). Furthermore, changes in crop yield after vegetable residues incorporation were also influenced by a combination of factors including species, production techniques, and soil fertility.

Conclusion

In summary, vegetable residues incorporation increased soil fertility and microbial diversity, optimized the cucumber root growth environment, and stimulated root vigor, thereby promoting cucumber plant growth and yield. The effects of different types of residues on improving soil properties were ordered sweet pepper plant residues > cucumber plant residues > tomato plant residues, and 20% of sweet pepper plant residues incorporation had the most significant effect on increasing crop yield.

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Not applicable.

Authors' contributions
Xiaoya Li designed research and experiments. Xiaotian Li and Jie Lou assisted in the completion of the study. Xiaolu Chen wrote the main manuscript text. Dalong Zhang revised the manuscript. Min Wei performed critical reading and revising suggestions. All authors read, reviewed, and approved the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This article does not contain any studies with human participants or laboratory animals.

Consent for publication
All the authors have approved the manuscript that is enclosed.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]College of Horticultural Science and Engineering, Shandong Agricultural University, Tai'an, Shandong, People's Republic of China. [2]Scientific Observing and Experimental Station of Environment Controlled Agricultural Engineering in Huang-Huai-Hai Region, Ministry of Agriculture and Rural Affairs, Tai'an, Shandong, People's Republic of China. [3]Shandong Collaborative Innovation Center for Fruit & Vegetable Production with High Quality and Efficiency, Tai'an, Shandong 271018, People's Republic of China.

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