



# Diversified crop rotation improves continuous monocropping eggplant production by altering the soil microbial community and biochemical properties

Muhammad Imran Ghani · Ahmad Ali · Muhammad Jawaad Atif · Shamina Imran Pathan · Giacomo Pietramellara · Muhammad Ali · Bakht Amin · Zhihui Cheng

Received: 10 August 2021 / Accepted: 15 July 2022 / Published online: 6 August 2022  
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

## Abstract

**Background and aims** Vegetable production under the plastic tunnel is a steadily growing sector worldwide, but this type of cultivation threatens environmental sustainability by degrading soil through continuous cropping conditions (CMC). This study aimed to assess the role of crop rotation (CR) with different winter leafy vegetables in ameliorating CMC problems by manipulating soil chemical and biological properties.

**Method** Four different leafy vegetables, including Welsh Onion (WO), Celery (CL), No Heading Chinese Cabbage (NCC), Lettuce (LT), and fallow eggplant (FE), were introduced during the eggplant fallow period (November–March) in two consecutive years, i.e., 2017 and 2018 following eggplant. We assessed eggplant production, soil chemical properties and described the soil microbial community under the introduced rotation system.

**Results** The results revealed that CR with winter leafy vegetables modified the soil environment by improving soil organic carbon (SOC), soil chemical, and biochemical characteristics in both years. Sequencing results showed significant variations in fungal and bacterial community structures at the genus and phylum levels in response to CR. CR reduced some disease-causing pathogens at the fungal genus levels, including *Fusarium* and *Ascomycota*, in both years and increased the abundance of some beneficial taxa such as *Mortierella* and *Bacillus*.

**Conclusion** These findings revealed the significance of crop rotation systems for sustainable production of eggplant under the plastic tunnel by enhancing soil physicochemical properties and soil beneficial microbes and reducing certain disease-causing soilborne pathogens.

Responsible Editor: Jan Jansa.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11104-022-05606-y>.

M. I. Ghani · A. Ali · M. J. Atif · M. Ali · B. Amin · Z. Cheng (✉)  
College of Horticulture, Northwest A&F University, Yangling 712100, China  
e-mail: chengzh@nwfau.edu.cn

M. I. Ghani  
College of Natural Resource and Environment, Northwest A&F University, Yangling 712100, China

M. J. Atif  
National Agricultural Research Centre, Horticultural Research Institute, Islamabad 44000, Pakistan

S. I. Pathan · G. Pietramellara  
Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50144 Florence, Italy

**Keywords** Soil microbial community · Crop rotation · Winter vegetables · Soil enzyme activities · Soilborne pathogen suppression

## Introduction

Vegetable production under plastic tunnels has become popular among farmers because of higher economic returns and has gradually expanded worldwide and reached up to 2 million ha; China contributes 85% of its total production area (Scarascia-Mugnozza et al. 2011; Zhang et al. 2015). Cultivation under a protected environment is often intensified with the repetition of the same crop species year after year, characterized as continuous mono-cropping (CMC) (Hu et al. 2017; Wang et al. 2015; Wang et al. 2014). However, concern has developed over the long-term sustainability of this cultivation system because excessive use of synthetic fertilizers and agrochemicals application under CMC is common, leading to continuous cropping obstacles (Li et al. 2017; Liu et al. 2019; Scarascia-Mugnozza et al. 2011). Soil hardening, nutrient imbalance, acidification, salinity, and autotoxicity have been reported as the leading causes of continuous cropping obstacles in the mono-cropping production system (Pervaiz et al. 2020). Eggplant (*Solanum melongena*) is an economically significant cash crop which is always cultivated under the plastic tunnel in China. Eggplant under plastic tunnels is continuously cultivated because of higher market demand and limited cultivated area (Wang et al. 2015). In addition, consecutive cultivation of vegetables under plastic sheds increased the income of farmers by more than 20 times per hectare compared to open field cultivation (Zhang 2021). The plastic tunnel has reported continuous cropping obstacles problems which have become a major constraint (Ghani et al., 2019a; Ghani et al., 2019b; Wang et al. 2015). Therefore, eggplant's continuous cropping obstacles are more severe and have become a major constraint for the sustainable production of eggplant (Ghani et al. 2022; Ghani et al. 2019a; Li et al. 2017). Thus, it becomes imperative to overcome continuous cropping obstacles of protected eggplant by improving the sustainability of soil management practices, soil ecology, and maintaining productive yield of continuous cropping eggplant.

Soil microbial communities are key to providing multiple ecosystem services to soils and are widely known as vital regulators of plant and environmental outcomes of agroecosystems (Bissett et al. 2013; Brennan and Acosta-Martinez 2017; Van Der Heijden et al. 2008). Previous empirical studies have reported

that the destruction of soil microbial diversity because of CMC is one of the key mechanisms behind continuous cropping obstacles (Ali et al. 2019a; Ali et al. 2019b; Ding et al. 2018; Ghani et al. 2019). Rhizosphere soil is the most vulnerable to various land-management practices, including planting regimes, fertilization, and irrigation relative to bulk soil (Strock et al. 2004). Different functional microbial groups, such as plant pathogens and beneficial organisms, could also influence the activity and diversity of soil microbial communities (Brussaard et al. 2007). Previously, researchers have reported that microbial communities are responsible for many biological processes and vital for soil health and the suppression of many soilborne disease development (Garbeva et al. 2004; Raaijmakers et al. 2009). For example, many studies have stated that CMC decreased several microbial species, contributing to the prevalence of soilborne diseases and reduced organic matter content in the soil (Fu et al. 2017; Li et al. 2014).

Considering the concerns mentioned above, one alternative to overcome these problems is incorporating cover crops into crop rotation during the fallow period. Many previous studies have reported that different cover crops have been introduced to achieve multiple benefits for crop production, soil quality, and soil ecology (Finney et al. 2017; Logsdon et al. 2002; López-Bellido et al. 2006). Diversification of cover crops with attuned niches plays a pivotal role in regulating the ecosystem by improving and conserving the soil quality, carbon sequestration, decomposition of organic matter, regulating the supply of soil nutrients, soil enzymatic activity, suppressing the soilborne pathogen, and increasing beneficial microbes (Finney et al. 2017; McDaniel et al. 2014). Cover crops augment C provisioning and facilitate the soil microbial community via unharvested residues and during their active growth as living crops through fine root turnover and root exudation (Finney et al. 2017; Kong and Six 2012). The inclusion of such practices can result in a higher abundance and diversity of soil microbes (Brennan and Acosta-Martinez 2017; Daryanto et al. 2018; Thapa et al. 2021). Plant roots and soil microbial communities have an inevitable relationship (Mendes et al. 2015). Previous studies have shown that cover crops and residue retention (above and belowground) have a considerable effect on soil microbial activity, population, and biomass, as well as the availability of nitrogen in intensive production

systems (Guo et al. 2008; Motisi et al. 2009; Tian et al. 2010; Tian et al. 2011). However, the effect of incorporation of belowground crop residue, i.e., dead root inputs, on soil microbial diversity has not been studied extensively. Furthermore, living cover crops or cover crop species-specific effects on the diversity and composition of microbial communities, as well as the time frame in which these effects occur, are still unknown (Finney et al. 2017). Therefore, it is imperative to know how cover crop selection shapes microbial communities to build efficient and effective management practices to restore healthy microbial communities in the agroecosystem (Finney et al. 2017; Lehman et al. 2015).

Plants can alter soil microbial communities by exuding different chemical compounds into the soil, and as a result, different species of plants leave different legacies in the soil, which further affects the growth of succeeding crops, known as plant-soil feedback (Heinen et al. 2018; Pathan et al. 2020; Philippot et al. 2013a). Even though evidence regarding plant species-specific influence on the soil is well accepted, there are minimal reports that have focused explicitly on living cover crop stands legacy effect on soil microbes (Hannula et al. 2021; Lehman et al. 2012; Pathan et al. 2020). Many studies have explored the relationship between soil microbial communities and cover crop residue legacy effects, the outcomes of which may be affected by dynamic interaction among cover crops and various factors of management, such as tillage and amendments that affect microbial communities (Frasier et al. 2016; Lienhard et al. 2014; Wortman et al. 2013). These outcomes are encouraging, but a deeper understanding of the short-term impacts of cover crops on the microbial community structure and function is still needed; cover crops can augment belowground plant inputs like plant root exudates containing C compounds, and these inputs are resulting in higher microbial efficacy and development (Strickland et al. 2015). Therefore, it is of paramount importance to propose such a study that isolates the crop-specific effect on soil microbial population from other prominent management factors to build our understanding of the interaction between living cover crops and soil microbes (Finney et al. 2017, 2017).

The main objectives of this study were to assess (1) how different cover crops could influence the structure and diversity of soil microbial communities as

well as soil physicochemical properties and (2) what distinctive soil edaphic factors are related to the key microbial changes. Thus, we hypothesized that successful integration of leafy winter vegetables would result in a significant shift in microbial community structure and improve soil biological health compared to fallow via positive plant-soil feedback (e.g., increasing synergistic interaction of belowground partners and reducing the abundance of antagonists). Moreover, since soil microbial communities are susceptible to soil environmental factors, we hypothesized that changes in soil microbial communities are due to the changes in soil physicochemical properties (e.g., pH, microbial biomass, enzyme activities). Finally, we proposed that the legacy effects of these cover cropping practices would potentially serve as a more efficient solution for sustainable eggplant production and soil management under plastic tunnel cultivation in North China.

## Material and methods

### Field description and experimental setup

A two-year field trial under a plastic tunnel consisted of four different vegetable crop rotations and fallow eggplant (1) Welsh onion - WO (*Allium fistulosum* L.) (2) Celery - CL (*Apium graveolens* var. *secalinum*) (3) No Heading Chinese Cabbage - NCC (*Brassica rapa*), (4) Lettuce - LT (*Lactuca sativa* L.) and (5) monocropping eggplant used as fallow eggplant (FE) were performed at Horticultural Research Farm of Northwest A&F University Yangling, China in the years 2017 and 2018, on Sandy loam soil, with a pH of 7.64 and EC of 148.64. Soil contained 19.18 g.kg<sup>-1</sup> soil organic carbon, total nitrogen 0.94 g.kg<sup>-1</sup>, total phosphorus 980 mg.kg<sup>-1</sup>, and 11,200 mg.kg<sup>-1</sup> total potassium, and available N, P, K 55.30 mg.kg<sup>-1</sup>, 28.67 mg.kg<sup>-1</sup> and 375.25 mg.kg<sup>-1</sup> respectively. Detailed information on how these measurements were done is provided in the supplementary material (Supplementary methods). The climate of the study area is cold semi-arid (steppe), with an annual average temperature of 14 °C and an average annual rainfall of 544 mm. During the winter, the temperature drops down to - 4 °C and remains around 4 to 6 °C inside the plastic tunnels. All four selected vegetables were selected based on their ability to tolerate frost during

cold winter. Further, these winter vegetables tend to uptake soil nutrients and provide them to succeeding crops (Ali et al. 2021). These leafy vegetables capture residual nitrogen and phosphorus in the soil and transfer them to subsequent crops, contributing to reduced nitrate leaching during the fallow period (Kanders et al. 2017; Xiao et al. 2021). In 2017 and 2018, experiments were organized in randomized block design and repeated for two consecutive years on the same plots with 3 replications. There were three beds in each plot, and the size of each plot and bed was 12.60 m<sup>2</sup> (3.6 m long × 3.5 m wide) and 4.20 m<sup>2</sup> (3.5 m long × 1.2 m wide), respectively. We established four different winter leafy vegetables as cover crops. Therefore, our experiment consisted of five treatments, as mentioned above. Each plot was separated by inserting a plastic sheet in the soil at a depth of approximately 50 cm and extended 5 cm above the ground to stop the movement of nutrients and water and also to prevent the mutual influence among different treatments. Cover crops were planted in each plot, excluding the FE, which was kept bare until the next cultivation period of eggplant. Cover crops were sown on 15 November 2017 and 19 November 2018 during the eggplant fallow period and were manually harvested between 1st to 5th of March each year. The root residues were left on field plots prior to eggplant plantation. After harvesting, each of the five plots was manually prepared for the main crop (eggplant) cultivation. Uniform eggplant seedlings cv. Tai Kong Qie Wang with three leaves was transplanted with a double row in each bed. The distance between rows was 80 cm and between plants was 50 cm; each row contained seven seedlings, and fourteen seedlings were transplanted in each bed. Further, before eggplant cultivation season (2017 and 2018), each plot was amended with 750.65 kg ha<sup>-1</sup> of organic fertilizer (PengDiXin) containing 30.03% organic matter, 2.55% N, 2.58% P, and 1.23% K and trace elements along with “SaKeFu” (119.04 kg ha<sup>-1</sup>), compound fertilizer consist of NPK (14.76%, 14.66%, and 14.46%, respectively), organic matter 2.4% and humic acid 5.2% used as a top dressing. JinBa, a compound chemical fertilizer containing N 16.94%, P 0.06%, K 3.46%, organic matter 2.50%, and humic acid 5.27% and often used locally for vegetable production, was used as a top dressing. Detailed information on how these measurements were done and the nutrient and carbon content of each fertilizer is provided in the

supplementary material (Supplementary methods). No fertilizer was applied during the winter leafy vegetable cropping season.

### Soil sampling

Before harvesting leafy vegetables in both years, soil sampling was done to elucidate the effect of various winter leafy vegetable cropping systems on soil nutrients and microbial communities during 2017–2018. In brief, five different plants in each bed were pulled carefully at the maximum leaf growth stage, and rhizosphere soil was collected by shaking the roots of leafy vegetable plants. The remaining soil was collected by gently brushing roots with the sterilized brush. The soil from the replicated plot was pooled together to make a composite sample. Bulk soil was collected from the control plot as a control treatment. Thus, there were three biological replicates from each cropping system. In total, fifteen soil samples were obtained from the study site, and soil samples were transported to the laboratory on ice. After sieving soil (2 mm sieved), half of the soil was stored at -80 °C for DNA extraction and further molecular analysis. The other half of the soil was air-dried and further used for soil physicochemical analysis.

### Soil physicochemical properties

Soil pH (5 g soil) and electrical conductivity (EC) were determined by pH meter and microprocessor conductivity meter 1:5 ratios (w/v), respectively. Soil organic carbon was estimated by the wet oxidation method (Nelson et al. 1979). For the estimation of available nitrogen (AN), we have used the alkali-hydrolyzed diffusion method of Shi (1996). Soil available phosphorus (AP) was determined by the creation of the blue-colored complex after the extraction with 0.5 M NaHCO<sub>3</sub> (pH 8.5) (Murphy and Riley 1962). Available K was determined through the extraction of 1 N ammonium acetate followed by emission spectroscopy (Knudsen et al. 1983).

### Soil microbial biomass

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were evaluated by chloroform fumigation and direct extraction process (Vance et al. 1987). The microbial biomass was calculated

using a conversion factor of 0.45 for MBC and 0.54 for MBN (Jenkinson and Ladd 1981).

#### Soil enzyme activities

Invertase activity was assessed using the previously described method (Tabatabai 1994). Urease and phosphatase activities were determined by using methods proposed by Tabatabai and Bremner (1972). The  $\beta$ -glucosidase activity was determined by the method proposed by Eivazi and Tabatabai (1988).

#### Measurement of eggplant yield

Eggplant yield was recorded as the first fifteen harvests of market-size fruits from each plot and measured in  $\text{kg m}^{-2}$ , and it was presented as an average value of each cropping system ( $\text{kg h}^{-1}$ ).

#### Soil DNA extraction and PCR amplification

Total soil DNA was extracted in triplicate from all samples using E.Z.N.A. soil DNA Kit (Omega Biotek, Norcross, GA, USA), following the recommended protocol of the manufacturer. The integrity and concentration of the DNA were evaluated by using a spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The 16S rRNA V4-V5 regions were amplified using 515F (GTGCCAGCMGCCGCGG) and 907R (CCGTCAATTCMTTTRAGTTT) (Lai et al. 2016) primers. Similarly, the fungal internal transcribed spacer (ITS) region of 18S rRNA was amplified using ITS1F as a forward primer (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R as a reverse primer (5'-GCTGCGTTCTTCATCGATGC-3') (Adams et al. 2013). All PCR amplifications were performed in triplicate 20  $\mu\text{L}$  of PCR reaction. The reaction mixture contained a hot master mixture with 4  $\mu\text{M}$  of 5 $\times$ FastPfu Buffer, 2  $\mu\text{L}$  of 2500  $\mu\text{M}$  dNTPs, 0.8  $\mu\text{L}$  of each primer (5  $\mu\text{M}$ ), 0.4  $\mu\text{L}$  of FastPfu Polymerase, and 10 ng of template DNA.

PCR running conditions for 16S rRNA gene were as follows: Initial denaturation of DNA at 95  $^{\circ}\text{C}$  for 2 min, followed by 25 cycles at 95  $^{\circ}\text{C}$  for 30 s, 55  $^{\circ}\text{C}$  for 30 s, elongation at 72  $^{\circ}\text{C}$  for 30s, and finally, a final extension of 5 min at 72  $^{\circ}\text{C}$ . PCR running conditions for the ITS region were: Initial denaturation of DNA at 95  $^{\circ}\text{C}$  for 2 min, followed by 25 cycles at 95  $^{\circ}\text{C}$  for 30 s, annealing for 45 s by lowering

the temperature from 64  $^{\circ}\text{C}$  to 55  $^{\circ}\text{C}$ , of elongation at 72  $^{\circ}\text{C}$  and finally terminal extension of 5 min at 72  $^{\circ}\text{C}$ . Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor<sup>TM</sup>-ST (Promega, U.S.). Purified amplicons were used for library preparation and sequencing, according to the Illumina 16S Metagenomic Sequencing Library Preparation guide (downloaded from [https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry\\_documentation/16s/16s-metagenomic-library-prep-guide-15,044,223-b.pdf](https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15,044,223-b.pdf)). Paired-end sequencing (2 $\times$ 300 bp) was carried out by using a MiSeq System (Illumina, CA, USA).

#### Bioinformatics analysis

The raw data were processed using QIIME (V1.9.1) pipeline under precise purified conditions. Filtered and valid tags were assembled, and chimeric sequences were detected and discarded using the UCHIME algorithm. After obtaining the quality sequences, the UPARSE clustering procedure was executed to assemble these quality sequences (97% sequence similarity) into operational taxonomic units (OTUs) (Edgar 2013). Taxonomic analysis of 16S rRNA and ITS genes was carried out through the SILVA (version 119; <http://www.arb-silva.de>) and UNITE (version 7.0; <http://unite.ut.ee/index.php>) databases for bacteria and fungi, respectively. The singletons were discarded before further analysis. Alpha- and beta-diversity (Bray-Curtis, UniFrac distance) indices were calculated using QIIME. Biomarker features among the sampling group were further screened by Metastat and LEfSe software. Differentially abundant bacterial and fungal OTUs taxa were calculated using linear discriminant analysis (LDA) effect size (LEfSe) analysis.

#### Statistical analysis

The statistical analyses were performed in R and IBM SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Soil biochemical properties, yield, and alpha diversity data were statistically analyzed by two-way ANOVA (cropping system  $\times$  year) to check any significant effect of cropping system, sampling year, and their

interaction on the variability of data, followed by one-way ANOVA with Tukey HSD test at 5% level to identify the impact of treatments. A Venn diagram was constructed based on unique and shared OTUs among treatments. PCoA (Principal coordinate analysis) based on Bray-Curtis similarity distance was applied to display the difference among samples' beta-diversity. The Pearson correlation was executed to examine which environmental factor strongly correlated with soil microbial class. UPGMA clustering analysis was used to exhibit the community composition difference. To identify significant differences among microbial communities based on different economic ranks, the LEfSe algorithm was performed (<https://huttenhower.sph.harvard.edu>).

## Results

### Soil physio-chemical properties

The effect of diversified crop rotation on different soil chemical properties is shown in Tables 1 and 2. Winter leafy vegetables showed a significant impact on soil pH and EC. In both years, soil pH increased significantly as compared to the mono-cropping system. In contrast, EC decreased considerably in all crop rotations compared to FE in both years, but

statistically, there is no significant difference in the two years of interaction. Soil organic carbon was higher in all crop rotations than in the mono-cropping system in both years, especially under NCC rotation. A similar trend was also observed for available nutrients. Available N (ammonia and nitrate), P, and K were higher in all crop rotations than in the mono-cropping system. The highest increase in soil available N was observed in NCC, followed by CL in 2018. The highest increase of macronutrients was observed in the second year (2018), with the highest value observed in NCC, followed by CL, WO, and LT. Sampling year did not show any significant difference between CR and mono-cropping systems.

Content of MBC and MBN were significantly increased under CR with winter leafy vegetables compared to the mono-cropping systems in both years (Table 3). The highest values of MBC ( $431.94 \text{ mg}\cdot\text{kg}^{-1}$  in 2017 and  $447.86 \text{ mg}\cdot\text{kg}^{-1}$  in 2018) and MBN ( $49.0 \text{ mg}\cdot\text{kg}^{-1}$  in 2017 and  $55.85 \text{ mg}\cdot\text{kg}^{-1}$  in 2018) were observed in NCC, followed by CL, WO, and LT.

A similar trend was also observed for soil enzyme activities. As for microbial biomass, all measured enzyme activities were significantly increased under CR with winter leafy vegetables compared to the mono-cropping system (Table 4). The highest values of all measured enzyme activities were reported in NCC, followed by CL, WO, and LT.

**Table 1** Influence of different diversified crop rotation systems on soil pH, EC, organic carbon. Main effect due to treatment (crop rotation), sampling year and their interaction was analyzed by Two-way ANOVA

Treatment	pH			EC ( $\mu\text{s} \cdot \text{cm}^{-1}$ )			Organic carbon ( $\text{g} \cdot \text{kg}^{-1}$ )		
	2017	2018	Means	2017	2018	Means	2017	2018	Means
One-Way ANOVA									
WO	$7.58 \pm 0.30^a$	$7.51 \pm 0.54^a$	$7.54^a$	$118.60 \pm 1.73^c$	$116.23 \pm 1.23^c$	$117.41^c$	$18.39 \pm 0.32^c$	$19.08 \pm 0.19^{bc}$	$18.37^{bc}$
CL	$7.50 \pm 0.32^a$	$7.43 \pm 0.49^a$	$7.47^a$	$106.13 \pm 1.98^d$	$104.01 \pm 1.10^d$	$105.07^c$	$19.34 \pm 0.02^{bc}$	$20.00 \pm 0.48^{ab}$	$19.68^b$
NCC	$7.64 \pm 0.42^a$	$7.56 \pm 0.18^a$	$7.60^a$	$119.33 \pm 3.38^c$	$116.95 \pm 2.12^c$	$118.14^c$	$21.07 \pm 1.10^{ab}$	$22.00 \pm 0.58^a$	$21.54^a$
LT	$7.56 \pm 0.02^a$	$7.48 \pm 0.82^a$	$7.52^a$	$165.33 \pm 3.40^b$	$162.02 \pm 1.97^b$	$163.67^b$	$18.08 \pm 0.22^c$	$18.42 \pm 0.29^c$	$18.25^{bc}$
FE	$7.22 \pm 0.37^b$	$7.29 \pm 0.16^b$	$7.25^b$	$273.00 \pm 1.95^a$	$281.19 \pm 1.70^a$	$277.10^a$	$17.72 \pm 0.24^c$	$16.83 \pm 2.15^c$	$17.28^c$
Year Means	$7.50^a$	$7.45^a$		$156.48^a$	$156.08^a$		$18.92^a$	$19.27^a$	
Two-Way ANOVA	Treatments	Year	Interaction	Treatment	Year	Interaction	Treatment	Year	Interaction
	***	ns	ns	***	ns	ns	***	ns	ns

Significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns: not significant. Multiple pairwise comparisons of means were done by Tukey's honestly significant difference (HSD) test at  $p < 0.05$  level of significance to analyze the individual effects of cropping system. Data represent means and errors of three replicates. Significant differences within columns are indicated in different lowercase letters. FE Fallow eggplant, NCC No Heading Chinese Cabbage, WO Welsh Onion, CL Celery, LT Lettuce

**Table 2** Influence of different diversified crop rotation systems on soil available nutrients. Main effect due to treatment (crop rotation), sampling year and their interaction was analyzed by Two-way ANOVA

Treatment	Available N (mg·kg <sup>-1</sup> )			Available P (mg·kg <sup>-1</sup> )			Available K (mg·kg <sup>-1</sup> )		
	2017	2018	Means	2017	2018	Means	2017	2018	Means
One-Way ANOVA									
WO	112.03 ± 1.43 <sup>c</sup>	119.67 ± 0.88 <sup>c</sup>	115.85 <sup>b</sup>	28.40 ± 1.21 <sup>cd</sup>	41.21 ± 0.87 <sup>ab</sup>	34.80 <sup>ab</sup>	347.37 ± 3.40 <sup>de</sup>	361.57 ± 3.87 <sup>bcd</sup>	354.47 <sup>b</sup>
CL	139.33 ± 1.82 <sup>b</sup>	145.17 ± 1.22 <sup>ab</sup>	142.25 <sup>a</sup>	27.39 ± 3.63 <sup>cd</sup>	39.68 ± 1.54 <sup>ab</sup>	33.54 <sup>bc</sup>	370.03 ± 6.30 <sup>abc</sup>	385.58 ± 5.14 <sup>a</sup>	377.81 <sup>a</sup>
NCC	142.33 ± 3.31 <sup>b</sup>	161.23 ± 1.92 <sup>a</sup>	151.78 <sup>a</sup>	25.40 ± 1.37 <sup>c</sup>	39.59 ± 1.97 <sup>ab</sup>	32.50 <sup>bc</sup>	365.87 ± 7.50 <sup>abcd</sup>	380.18 ± 3.63 <sup>ab</sup>	373.03 <sup>a</sup>
LT	110.13 ± 1.08 <sup>c</sup>	104.67 ± 0.65 <sup>c</sup>	107.40 <sup>b</sup>	31.62 ± 1.16 <sup>d</sup>	43.09 ± 2.43 <sup>b</sup>	37.36 <sup>a</sup>	342.46 ± 13 <sup>e</sup>	363.78 ± 3.87 <sup>bcd</sup>	353.12 <sup>b</sup>
FE	103.33 ± 1.37 <sup>c</sup>	108.179 ± 0.84 <sup>c</sup>	105.75 <sup>b</sup>	24.83 ± 0.25 <sup>d</sup>	37.61 ± 1.18 <sup>d</sup>	31.21 <sup>c</sup>	341.72 ± 5.41 <sup>e</sup>	356.14 ± 4.04 <sup>cde</sup>	348.93 <sup>b</sup>
Year means	120.34 <sup>b</sup>	128.87 <sup>a</sup>		27.53 <sup>b</sup>	40.35 <sup>a</sup>		353.49 <sup>b</sup>	369.45 <sup>a</sup>	
Two-Way ANOVA	Treatments	Year	Interaction	Treatment	Year	Interaction	Treatment	Year	Interaction
	***	ns	ns	***	***	ns	***	*	ns

Significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns: not significant. Multiple pairwise comparisons of means were done by Tukey's honestly significant difference (HSD) test at  $p < 0.05$  level of significance to analyze the individual effects of cropping system. Data represent means and errors of three replicates. Significant differences within columns are indicated in different lowercase letters. FE Fallow eggplant, NCC No Heading Chinese Cabbage, WO Welsh Onion, CL Celery, LT Lettuce

**Table 3** Influence of different diversified crop rotation systems on soil MBC and MBN. Main effect due to treatment (crop rotation), sampling year and their interaction was analyzed by Two-way ANOVA

Treatment	MBC (mg·kg <sup>-1</sup> )			MBN (mg·kg <sup>-1</sup> )		
	2017	2018	Means	2017	2018	Means
One-Way ANOVA						
WO	338.42 ± 5.15 <sup>c</sup>	351.03 ± 3.00 <sup>c</sup>	344.72 <sup>c</sup>	32.16 ± 1.09 <sup>de</sup>	34.63 ± 1.85 <sup>d</sup>	33.40 <sup>c</sup>
CL	412.06 ± 5.32 <sup>b</sup>	417.37 ± 3.82 <sup>b</sup>	414.71 <sup>b</sup>	38.74 ± 1.37 <sup>cd</sup>	45.87 ± 1.62 <sup>bc</sup>	42.31 <sup>b</sup>
NCC	431.94 ± 3.40 <sup>ab</sup>	447.86 ± 3.19 <sup>a</sup>	439.90 <sup>a</sup>	49.00 ± 3.15 <sup>ab</sup>	55.86 ± 2.75 <sup>a</sup>	52.43 <sup>a</sup>
LT	335.74 ± 4.20 <sup>c</sup>	344.44 ± 6.25 <sup>c</sup>	340.09 <sup>c</sup>	29.46 ± 1.17 <sup>def</sup>	35.44 ± 1.95 <sup>d</sup>	32.45 <sup>c</sup>
FE	236.02 ± 3.22 <sup>d</sup>	247.95 ± 2.83 <sup>d</sup>	241.99 <sup>d</sup>	20.64 ± 1.93 <sup>f</sup>	24.12 ± 1.64 <sup>ef</sup>	22.38 <sup>d</sup>
Year Means	350.84 <sup>b</sup>	361.73 <sup>a</sup>		34.00 <sup>b</sup>	39.18 <sup>a</sup>	
Two-Way ANOVA	Treatments	Year	Interaction	Treatment	Year	Interaction
	***	***	ns	***	***	ns

Significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns: not significant. Multiple pairwise comparisons of means were done by Tukey's honestly significant difference (HSD) test at  $p < 0.05$  level of significance to analyze the individual effects of cropping system. Data represent means and errors of three replicates. Significant differences within columns are indicated in different lowercase letters. FE Fallow eggplant, NCC No Heading Chinese Cabbage, WO Welsh Onion, CL Celery, LT Lettuce

## Alpha diversity

After quality filtering, chimera cleaning, and removal of singletons, 18,266 and 47,824 reads were identified for bacterial 16S rRNA gene and internal transcribed spacer (ITS) fungal region across all samples. In the bacterial population, we identified 4558 OTUs, classified into 37 phyla, 93 classes, 213 orders, 405 families, 785 genera, and 1570 species. A total of 1053 OTUs were obtained in the fungal population, classified into nine phyla, 25 classes, 61 orders, 120 families, 236 genera, and 375 species

across all sampling. The rarefaction curve was used to evaluate OTUs richness between bacteria and fungi, indicating the sequencing depth sufficient to cover detectable species in all samples (Fig. S1). Exclusive and shared OTUs between different cropping systems were revealed through the Venn diagram (Fig. S2). In 2017 total of 243 fungal OTUs were shared between all cropping systems. The highest exclusive OTUs (67, 27.57%) were found in both FE and NCC, and the highest shared OTUs (146, 60.08%) were found between FE-WO and FE-CL. In 2018, 275 fungal OTUs were shared among all

**Table 4** Influence of different diversified crop rotation systems on soil enzyme activities. Main effect due to treatment (crop rotation), sampling year and their interaction was analyzed by Two-way ANOVA

Treatment	Soil Invertase Activity (Glucose mg g <sup>-1</sup> )			Soil Urease activity (NH <sub>4</sub> -N mg g <sup>-1</sup> )			β-glucosidase (mg p-nitro phenyl kg <sup>-1</sup> soil h <sup>-1</sup> )			Soil phosphatase (P <sub>2</sub> O <sub>5</sub> mg·100 g <sup>-1</sup> )		
	2017	2018	Means	2017	2018	Means	2017	2018	Means	2017	2018	Means
WO	55.35 ± 0.84 <sup>d</sup>	57.54 ± 1.96 <sup>cd</sup>	56.45 <sup>c</sup>	4.54 ± 0.09 <sup>cd</sup>	5.03 ± 0.38 <sup>abcd</sup>	4.79 <sup>b</sup>	434.49 ± 7.28bc	440.37 ± 2.40bc	437.43b	29.12 ± 1.09	30.66 ± 1.16	29.89abc
Celery	61.16 ± 2.71 <sup>bc</sup>	64.37 ± 1.83 <sup>ab</sup>	62.77 <sup>b</sup>	5.35 ± 0.16 <sup>abcd</sup>	6.21 ± 0.93 <sup>ab</sup>	5.78 <sup>a</sup>	515.35 ± 6.25a	527.54 ± 6.90a	521.45a	29.95 ± 3.35	35.15 ± 1.32	31.05ab
NHCC	65.37 ± 1.84 <sup>ab</sup>	69.67 ± 2.73 <sup>a</sup>	67.52 <sup>a</sup>	5.77 ± 0.44 <sup>abc</sup>	6.34 ± 0.63 <sup>a</sup>	6.05 <sup>a</sup>	532.04 ± 4.28a	546.34 ± 7.03a	539.19a	33.68 ± 1.26	35.12 ± 2.33	34.40a
Lettuce	55.78 ± 1.90 <sup>cd</sup>	56.72 ± 3.20 <sup>cd</sup>	56.25 <sup>c</sup>	5.18 ± 0.50 <sup>abcd</sup>	5.39 ± 0.41 <sup>abcd</sup>	5.28 <sup>ab</sup>	450.78 ± 3.98b	460.72 ± 11.08b	455.75b	26.94 ± 1.82	27.67 ± 1.87	27.30bc
FE	44.58 ± 2.12 <sup>e</sup>	48.78 ± 1.62 <sup>e</sup>	46.68 <sup>d</sup>	4.25 ± 0.30 <sup>d</sup>	4.78 ± 0.62 <sup>bed</sup>	4.51 <sup>b</sup>	402 ± 4.56d	408.78 ± 6.70 cd	405.39c	24.77 ± 0.85	25.94 ± 0.72	25.35c
Year Means	59.42 <sup>a</sup>	56.45 <sup>b</sup>		5.02 <sup>b</sup>	5.55 <sup>a</sup>		466.93b	476.75a		28.89a	30.30a	
Two-Way ANOVA	***	*	ns	***	*	ns	***	*	ns	***	ns	ns

Significance: \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns: not significant. Multiple pairwise comparisons of means were done by Tukey's honestly significant difference (HSD) test at  $p < 0.05$  level of significance to analyze the individual effects of cropping system. Data represent means and errors of three replicates. Significant differences within columns are indicated in different lowercase letters. FE Fallow eggplant, NCC No Heading Chinese Cabbage, WO Welsh Onion, CL Celery, LT Lettuce

cropping systems. The highest unique OTUs (62, 25.83%) were observed in FE, while the highest shared OTUs (193, 80.41%) were found between CL and WO. The bacterial Venn diagram illustrated that 2088 bacterial OTUs were shared between different cropping systems and FE in 2017. The highest unique OTUs (104, 4.98%) were observed under FE, and maximum shared OTUs (647, 30.98%) were observed between NCC and WO. The total number of OTUs, 2090, was found in 2018. WO and NCC shared maximum OTUs (826, 39.52%) and maximum exclusive OTUs (172, 8.23%) under FE.

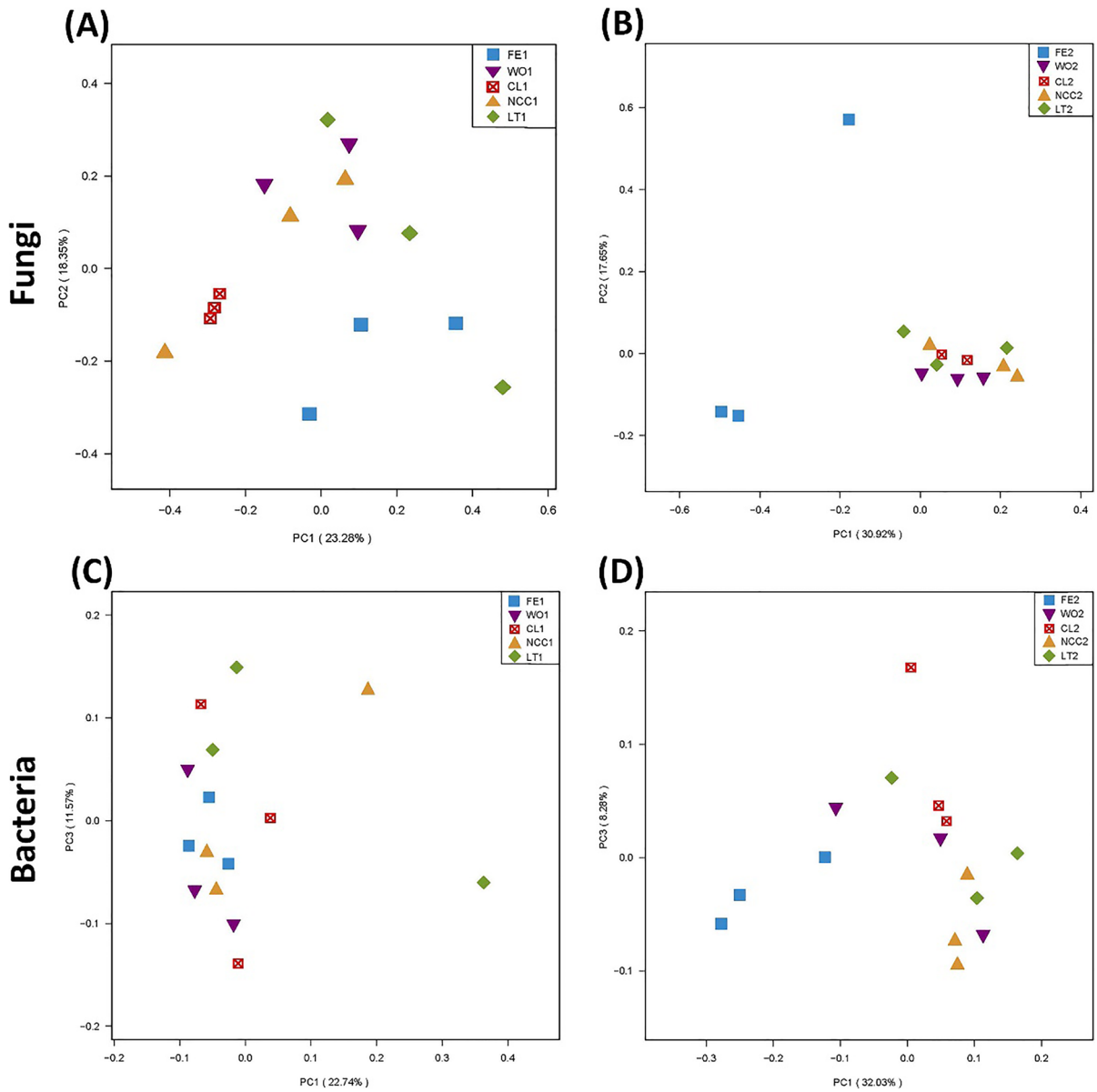
Estimated diversity indices for fungi and bacteria are shown in Tables S1 and S2. ACE and Chao indices were used to reflect the OTUs in different samples. In contrast, the Shannon index was used to reflect the richness and evenness of species in the sample (Table S1). In 2017, fungal diversity indices were higher in the mono-cropping system than in the CR with winter leafy vegetables. In contrast, diversity and richness were significantly higher in CR than in mono-cropping in 2018. A similar trend was observed for the bacterial community (Table S2). Fungal and bacterial diversities did not significantly impact the sampling year or their interaction with treatment.

### Beta diversity

PCoA of Bray-Curtis distance was used to analyze the variation in the microbial communities as affected by different cropping systems (Fig. 1). The first two components, which accounted for 56.63% (2017) and 48.57% (2018) of the total explained variance, were used to visualize an ordination biplot PC1 vs PC2 (Fig. 2). The plot revealed that the fungal community of FE (Fallow eggplant) was clustering separately from CR. However, fewer differences were observed among CR with winter leafy vegetables, especially in 2017. In 2018, a fungal community under CL plantation was clustering separately from other winter leafy vegetables.

In 2017, the 1st and 2nd axis showed a 41.59% variance in bacterial communities, while it depicted a 42.90% variation in 2018. No differences were observed during the 2017 sampling as communities were overlapping and there was no apparent clustering among them (Fig. 1).





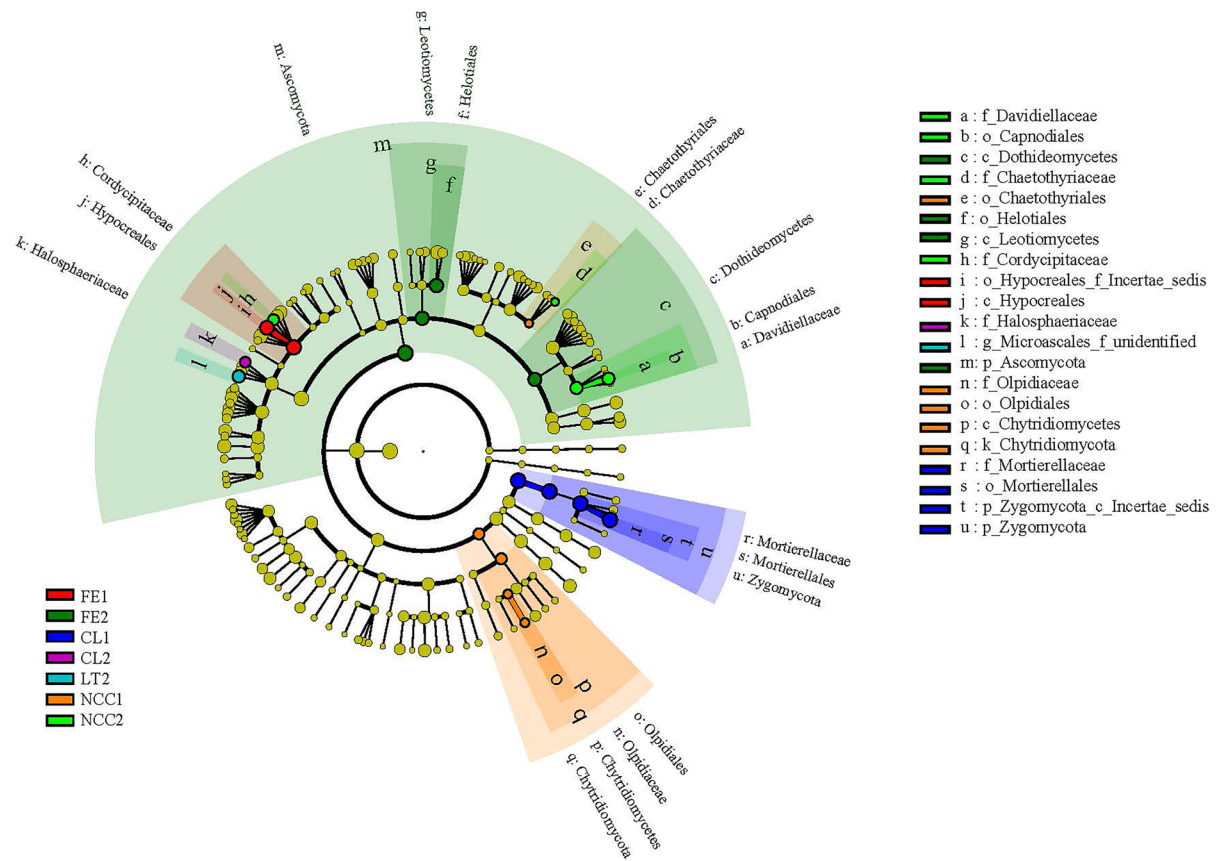
**Fig. 1** Principal coordinates analysis (PCoA) of soil fungal and bacterial communities at the OTU level among different diversified crop rotation. The fig a and b represent variance in soil fungal community structures and fig c and d represent var-

iance in soil bacterial community structures. 1: 2017, 2:2018, FE: Fallow eggplant, NCC: No Heading Chinese Cabbage, WO: Welsh Onion, CL: Celery, LT: Lettuce

However, some differences were observed in 2018. The fungal community showed that the mono-cropping (FE) bacterial community was clustering separately from different CR with winter leafy vegetables. Further, the bacterial community under CL and NCC were clustered independently from each other.

### Taxonomic composition

Taxonomic profiling revealed that fungal phyla in four cropping systems were similar in diversity while diverse in abundance in 2017 and 2018 (Fig. S3). Overall, six fungal phyla were identified, and four out of six accounted for 96% and 98% in



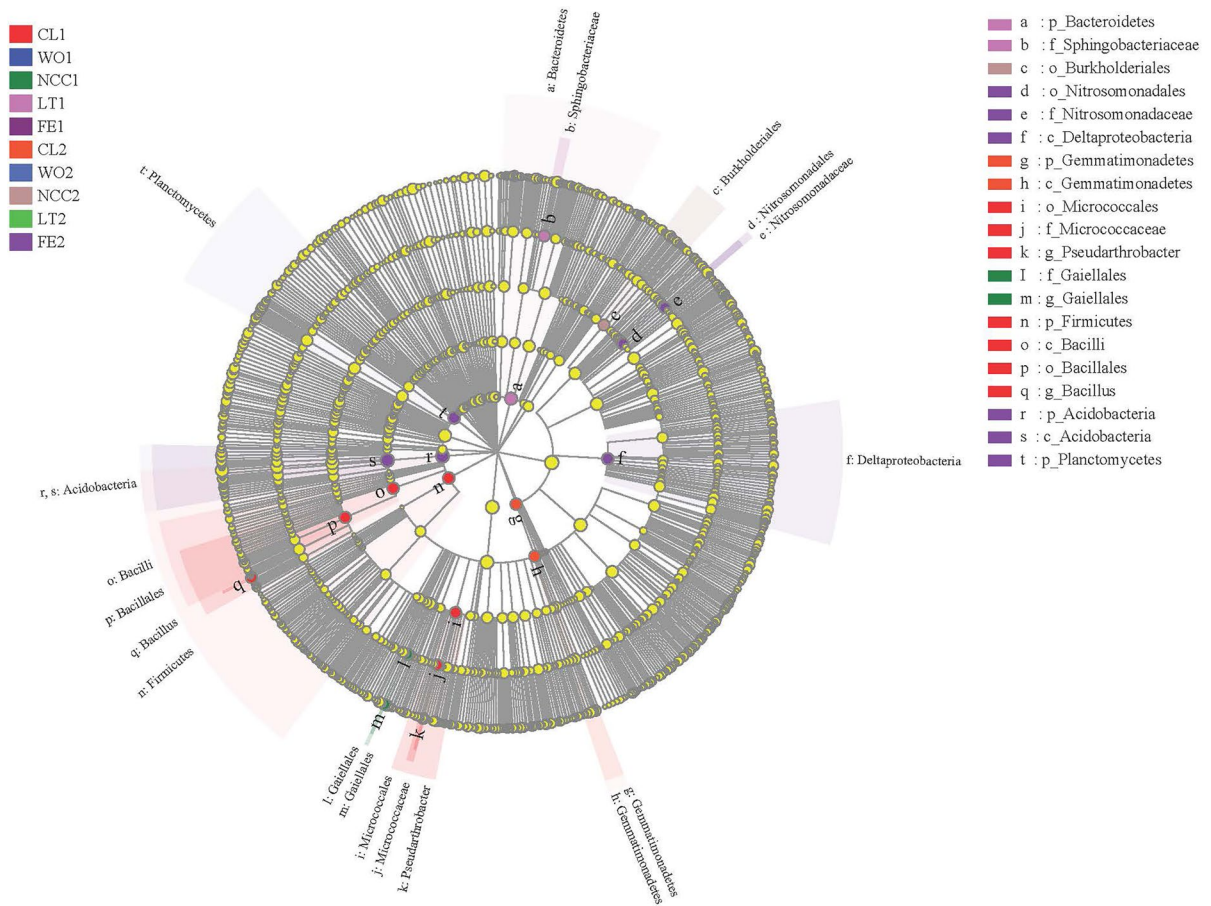
**Fig. 2** Linear discriminant analysis effect size (LefSe) for fungal taxa under diversified crop rotation. Cladogram showing significantly enriched fungal taxa (from phylum to family level). Significant differences are defined at  $P < 0.05$  and an LDA score  $> 4.0$ . Cladogram representing the taxonomic hierarchical structure of phylotype biomarkers identified under

2017 and 2018, respectively. *Ascomycota*, followed by *Zygomycota*, were the most abundant phyla in CR during both years (Fig. S3). There was a reduction in the relative abundance of *Ascomycota* in CR in both years, except for lettuce rotation only in the first year. The highest reduction was observed in CL (44.14%) in 2017. Relative abundance of *Basidiomycetes* was very low in all CR compared to monocropping except LT, where it increased by 20.53 and 2.73% in 2017 and 2018. At the genus level, *Mortierella* is followed by *Acremonium*, *Sordariomycetes*, *Hypocreales*, *Fusarium*, *Fungi\_unclassified*, *Botrytis*, *Ascomycota\_unclassified*, *Chaetomiaceae*, *Incertae\_sedis\_unclassified* were ten major fungal taxa found in both years (Fig. S4). CR systems

diversified crop rotation in two years. Yellow circles, phylotypes for which relative abundance is not significantly different under given treatments while other showed significantly affected by CR treatment and year. 1: 2017, 2:2018, FE: Fallow eggplant, NCC: No Heading Chinese Cabbage, WO: Welsh Onion. CL: Celery, LT: Lettuce

have a variable effect on the bacterial genus in both years.

In both years, different vegetable cropping systems altered bacterial communities at phylum and genus levels (Figs. S3 and S5). A total of 16 bacterial phyla were observed, and ten out of sixteen accounted for 96.14% in 2017 and 96.57% in 2018 (Fig. 3). *Proteobacteria* was the most abundant bacterial phyla, followed by *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, and *Nitrospirae* in both years. Relative abundance of *Proteobacteria* was increased in all CR systems in both years compared to monoculture except NCC only in 2017. The crop rotation system reduced the abundance of *Acidobacteria* compared to FE. The highest reduction was observed under LT



**Fig. 3** Linear discriminant analysis effect size (LEfSe) for Bacterial taxa under diversified crop rotation. Cladogram showing significantly enriched bacterial taxa (from phylum to family level). Significant differences are defined at  $P < 0.05$  and an LDA score  $> 4.0$ . Cladogram representing the taxonomic hierarchical structure of phylotype biomarkers identified

under diversified crop rotation in two years. Yellow circles, phylotypes for which relative abundance is not significantly different under given treatments while other showed significantly affected by CR treatment and year. 1: 2017, 2:2018, FE: Fallow eggplant, NCC: No Heading Chinese Cabbage, WO: Welsh Onion. CL: Celery, LT: Lettuce

and NCC in 2017 and 2018, respectively. CR systems had a positive effect on *Actinobacteria*, as there was an increase in the relative abundance in both years in all CR systems compared to FE. *Chloroflexi* was increased in 2017 except CL, which decreased to 5.87%. However, in 2018, *Chloroflexi* decreased in all CR systems except CL, increasing 2.5% compared with FE. *Planctomycetes* exhibited a declining trend in 2017 and 2018 in all CR systems compared with FE. *Bacteroidetes* and *Firmicutes* exhibited a declining trend in 2017 except in LT. On the contrary, their abundance increased in 2018 in all CR systems compared to FE except CL 6.13%. *Gemmatimonadetes* decreased in all cropping systems in both years except

in 2017; it was 6.92% and 24.94% higher in CL and NCC, respectively. In 2017, *Nitrospirae* decreased in all cropping systems compared to FE except in NCC. While there was a reduction during 2018, and the highest reduction of 38.02% was observed under WO. At genus level, the *Actinobacteria*, *Nitrospira*, and *Bacillus* were the most dominant in all the CR systems in both years. The dominant families (*Anaerolineaceae*, *Gemmatimonadaceae*, *Nitrosomonadaceae*) followed by the orders (*Xanthomonadales*, and *Gaiellales*), and the genera (JG30-KF-CM45, H16) were also enriched in all CR systems. CR systems have a variable effect on the bacterial genus in both years.

## Comparative assessment of microbial biomarkers

LFSE (linear discriminant analysis effect size) analysis and linear discriminant analysis (LDA) was employed to determine the further changes that occurred in fungal and bacterial community structure under CR systems (Fig. 2). LDA score higher than four was used to distinguish the different fungal and bacterial groups among various CR systems and years. Relative abundance of the fungal order *Hypocreales* was significantly enriched in FE compared to CR systems in 2017, while the relative abundance of *Dothideomycetes* and *Leotiomyces* was higher in FE in 2018 compared to CR systems. Relative abundances of the families *Mortierellaceae* and *Halosporiaceae* were significantly affected by CL plantation in 2017 and 2018, respectively. LT rotation significantly affected the relative abundance of order *Microascales* only in 2018. NCC cropping system significantly affected the *Helotiales* and *Leotiomyces* in 2017 and *Cordycipitaceae* in 2018.

For bacteria, the relative abundance of *Betaproteobacteria* (*Nitrosomonadaceae*) and *Deltaproteobacteria* was significantly changed in monoculture in 2017, while the relative abundance of *Acidobacteria* and *Planctomycetes* was changed in 2018 (Fig. 3). Genus *Pseudarthrobacter* (*Micrococcaceae*, *Actinomyces*) and family bacilli (*Firmicutes*) were significantly affected by CL rotation in 2017, while significant changes were only observed to *Gemmatimonadetes* in 2018 rotation. Taxa belonging to the *Gaiellales* order from *Actinonacteria* and *Comamonadaceae* family from *Betaproteobacteria* significantly responded to NCC rotation in 2017 and 2018, respectively. LT rotation only has a significant effect on *Sphingobacteriaceae* (*Bacterioidetes*) during 2017. Plantation of WO did not have any significant impact on any of bacterial taxa.

## Control of soil chemical and biochemical properties on soil bacterial and fungal community

Pearson correlation was executed among different soil edaphic factors to examine which environmental factor strongly correlates with soil bacterial and fungal taxon abundances (Figs. 4 and 5). Fungal taxa at the genus level showed a strong positive correlation, including *Mortierella*, with all soil properties (Fig. 4). The taxa *Sordariales* and *Rozellomycota*

positively correlated with pH, soil invertase, and  $\beta$ -glucosidase. *Verticillium* has a positive correlation with MBC, MBN, OM, SOC, and  $\beta$ -glucosidase. While some *Ascomycota\_unclassified* and *Microasaceae\_unidentified* had a negative correlation with all parameters.

Similarly, a significant positive correlation was observed among *Solirubrobacter*, MSB-1E8, TRA3–20, *Nocardioides*, KD4–96, and AP, MBN, MBC,  $\beta$ -glucosidase, invertase, OM, SO, AK, soil urease, and soil phosphatase (Fig. 5). In contrast, a highly negative correlation was observed between *Bacillus*, RB41, *Tectomicrobia*, TK10, *Acidimicrobiales*, *Gemmatimonadaceae*, *Armatimonadetes*, *Latescibacteria*, NB1-j, *Acidobacteria*, *Planctomycetaceae*, *Acidobacteria*, *Bryobacter*, *Roseiflexus*, H16, *Gaiellales* and *Actinobacteria*, and AP.

## Eggplant yield

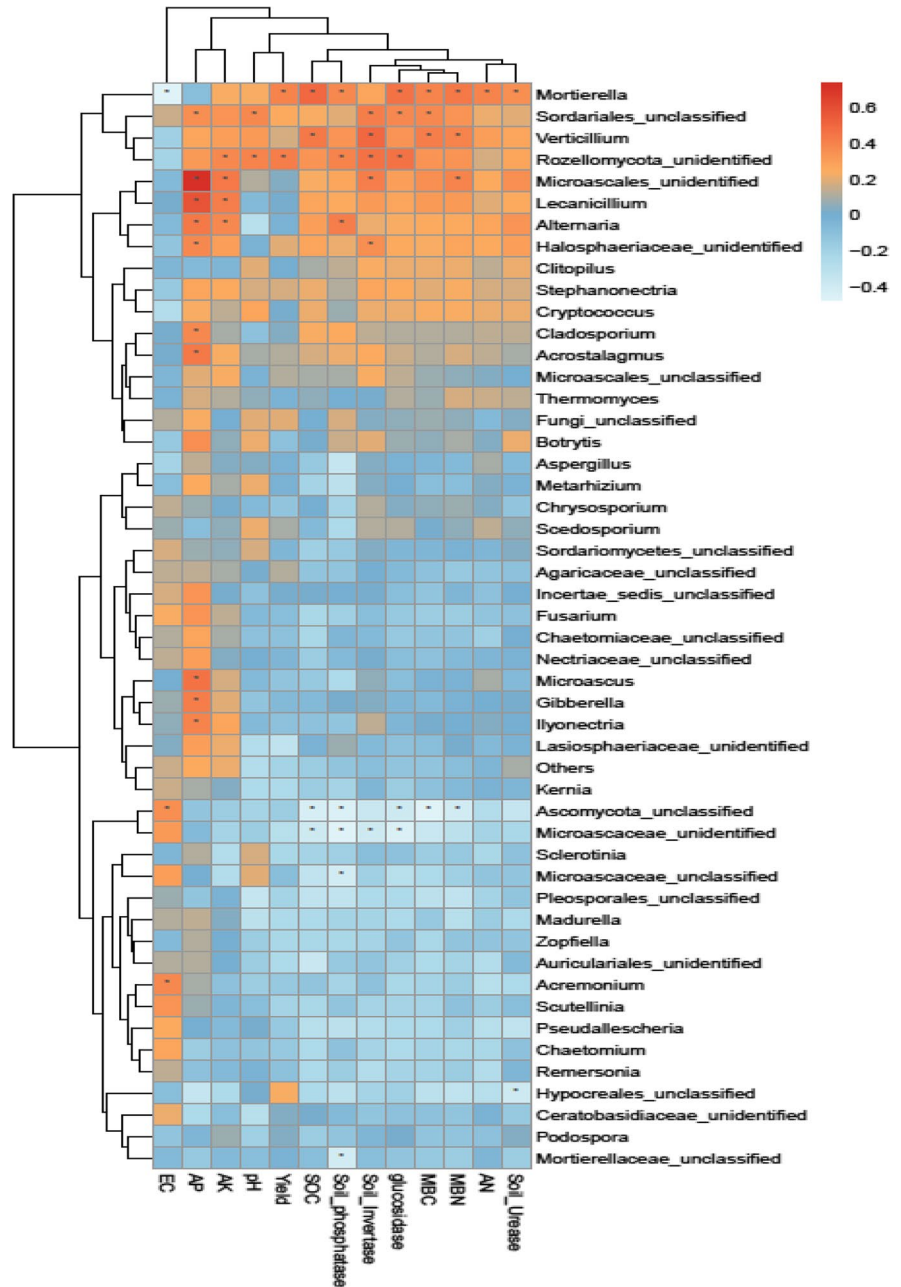
The effects of different leafy vegetable species on eggplant yield during the years 2017 and 2018 are shown in Fig. 6. Results showed that rotation with leafy vegetables significantly increases eggplant production compared with fallow eggplant. The highest production was observed under the NCC rotation treatment in both years.

## Discussion

### Effect of crop rotation with different winter vegetables on soil physicochemical properties

Cover crops enhance soil nitrogen availability by nutrient acquisition during their growth. Afterward, they are made available to subsequent crops by the residue decay process, hence reducing nitrate leaching (White et al. 2022). Several studies have indicated that belowground rhizodeposits and roots inputs are the primary sources of belowground C and contribute up to 75% of SOM (Gale and Cambardella 2000; Jones et al. 2009). Continuous rhizodeposition may promote microbial biomass carbon, a crucial precursor to SOM (Sokol and Bradford 2019). Further, previous studies showed that root-derived C was more important for the formation of stable C (Kong and Six 2010, 2012). Under this concept, NCC and CL increased SOC and MBC by releasing root exudates

**Fig. 4** Pearson correlation coefficients between fungal taxon at genus level and environmental factors. Values >0.55 or <0.55 are significant positive or negative correlation. Red to Blue colour indicates positive to negative correlations

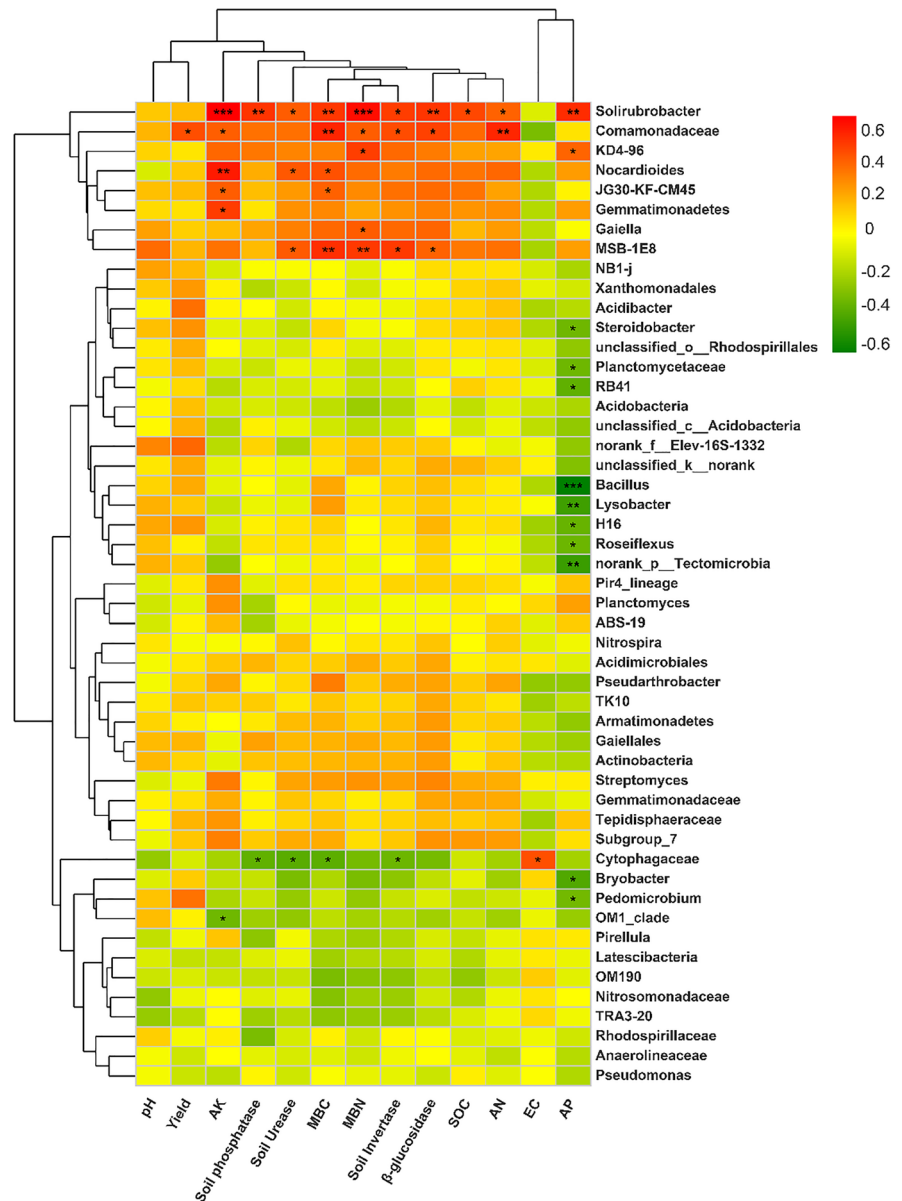


or through dead root residues. Previous studies indicated that living cover crops significantly improved SOC and MBC content compared to bare fallow by releasing low molecular C contents in the soil through rhizodeposition (Austin et al. 2017; Coombs et al. 2017). However, rhizodeposition decrease with plant maturity, but the addition of mature roots as residue to soil act as a microbial substrate, thus increasing

MBC and SOC (Bottner et al. 1988; Chahal and Van Eerd 2020).

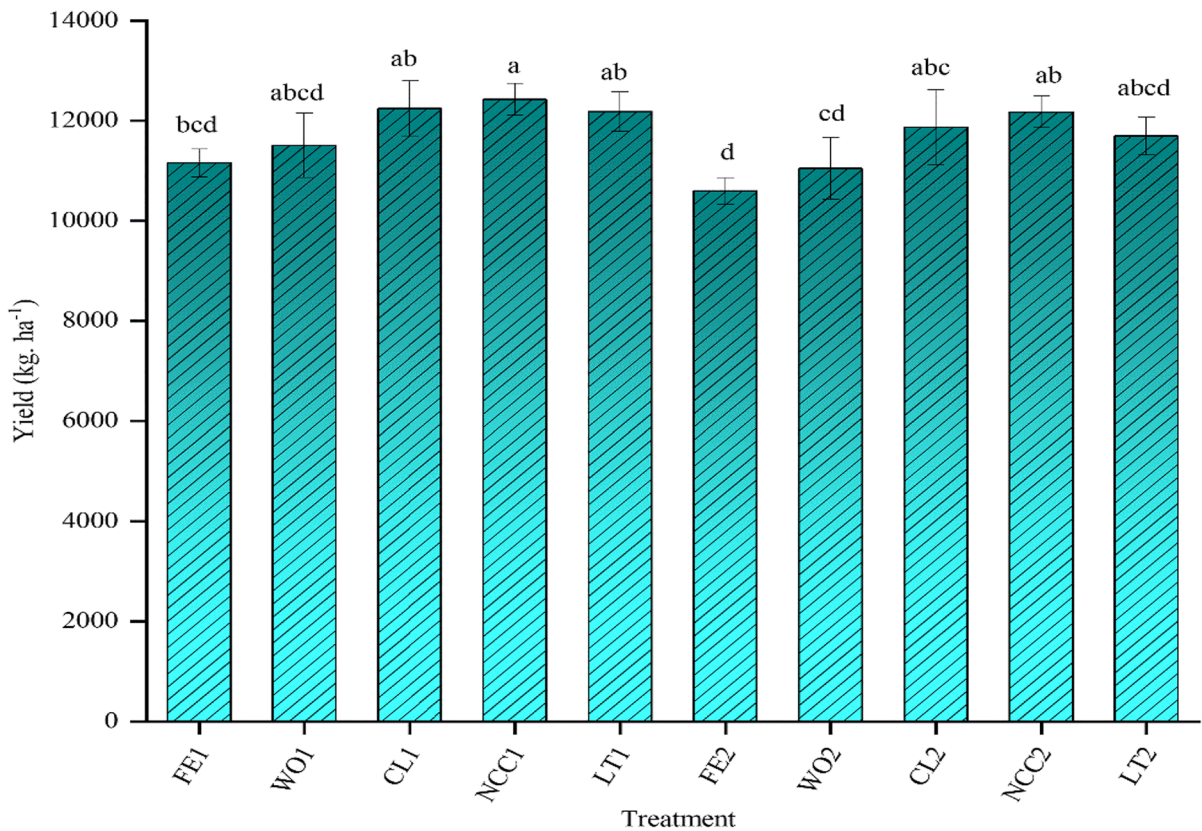
Soil nutrients and enzymatic activities are vital indicators for soil quality and belowground microbial activities. Leafy vegetables significantly induced soil nutrients and biological activities in the present study (Tables 1, 2 and 3). In both years, maximum available nutrients and biological activities were observed

**Fig. 5** Pearson correlation coefficients between bacterial taxon at genus level and environmental factors. Values  $>0.5$  or  $<0.5$  are significant positive or negative correlation. Red to green colour indicates positive to negative correlations



under the NCC and CL rotations. This could be due to the greater capacity of non-leguminous crops to uptake and conserve residual N, which afterward become available to subsequent cash crop through rhizodeposition or cover crop residue decomposition (Shipley et al. 1992; Stroock et al. 2004). Further, several previous studies have reported that root C and N inputs are mainly responsible for regulating soil microbial and enzyme activities (Cotrufo et al. 2013; Schmidt et al. 2011). Following this idea, NCC and CL modify the soil environment by releasing

nutrients into the soil through their roots in the form of root exudates or dead roots. Our findings were consistent with previous short-term observations that substantial improvements in soil properties may be predicted under different agronomic practices such as planting systems and soil quality (Chang et al. 2017; Wang et al. 2020). Our results exhibited that different cover crops could be effectively used in crop rotation by reducing external inputs without creating negative soil changes. Therefore, using CR with winter leafy vegetables effectively enhances the soil



**Fig. 6** Effect of different leafy vegetable species on eggplant yield during the years 2017 and 2018. Data are presented as means with standard deviation ( $n=3$ ). Different letters show

significant differences at  $p < 0.05$  level. FE: fellow eggplant; WO: welsh onion; CL: Celery; NCC: no heading Chinese cabbage; LT: Lettuce

physicochemical properties of continuous cropping soil under plastic greenhouse cultivation.

Effect of crop rotation with different winter vegetables on eggplant yield

CR with winter leafy vegetables reduced yield losses due to mono-cropping eggplant. NCC and CL significantly increased eggplant yield in both years. The results revealed that the higher yield of eggplant was associated with higher nutrient deposition and soil biological activation, which indicated the beneficial effect of these crop rotations in increasing crop yield and restitution of degraded soil due to continuous cropping of eggplant. Previous studies have demonstrated the yield-promoting impact of different crop rotations with spinach-cucumber and Chinese cabbage-tomato systems (Ali et al. 2021; Lyu et al. 2020). The other possible reason behind yield

enhancement in the NCC system is that Brassicaceae vegetables contain secondary metabolites such as glucosinolates, and their breakdown product (isothiocyanate) can suppress numerous soilborne pathogens such as *Fusarium oxysporum* of eggplant and hence, have a positive impact on eggplant yield (Li et al. 2017; Zhou et al. 2017).

Effect of crop rotation with different winter vegetables on soil microbial community structure and diversity

There were no differences observed in the structure of microbial communities (both fungi and bacteria, Fig. 1) between FE and CR systems during the first year of rotation (in 2017). However, changes were observed in 2018 (second year), as both fungal and bacterial communities were clustering separately under FE and CR systems. A previous study by

Ashworth et al. (2017) exhibited similar results where corn-soybean rotation showed similar communities after one season. This evidence indicates that various crop rotations may be used to modify soil microbes. This observation indicates that distinct communities were not crop-specific; rather, it depends on crop rotation duration. Similarly, in this study, bacteria and fungi communities' structure exhibited rotation-specific impact rather than crop-specific (Chamberlain et al. 2020).

Crop rotations do not have a significant effect on bacterial  $\alpha$ -diversity. It is not surprising that the effects are not strong in the short term but only apparent after more than one year because all of the plots in this study were managed similarly. These results are consistent with a meta-analysis done by Venter et al. (2016), indicating that microbial richness and diversity were associated with more diverse and long-term crop rotation. Different leafy vegetables can have a different impact on fungal  $\alpha$ -diversity in continuous mono-cropping soil. As shown in Table S1, in 2018, fungal richness was increased in CL, WO, and LT. Rotation of crops with strong allelochemicals can effectively influence the richness and diversity of soil fungi (Ding et al. 2018). It has been well documented that continuous mono-cropping can increase the soil fungal richness (Ghani et al. 2019b; Meng et al. 2012).

In this study, NCC rotation decreased fungi richness in both years. There are several explanations for this; NCC has different allelochemicals that can reduce eggplant self-toxicity, inhibit pathogenic fungi, and improve soil micro ecological environment (Alvey et al. 2003; Zhu and Fox 2003; Li et al. 2012). This is one of the important findings of this study because the rotation of different leafy vegetables has the opposite impact on fungal diversity and richness in mono-cropping soil.

#### Effect of crop rotation with different winter vegetables on soil microbial composition

Plants influence soil microbes through the magnitude and quality of root exudates (Philippot et al. 2013a) and litter (De Long et al. 2019; Hannula et al. 2021). Continuous monocropping of eggplant increased the accumulation of plant pathogens and disease incidence in the plant. Different cover crops included in crop rotation significantly decreased the abundance of pathogenic bacteria and fungi and increased beneficial

microbes (Liu et al. 2021). Still, this effect was crop and rotation specific due to the different nature of root exudates, litter quality, and chemistry. Leafy vegetables induced the abundance of several beneficial microbial phyla that could suppress plant pathogens and promote plant growth, such as *Proteobacteria*, *Planctomycetes*, *Firmicutes*, and *Bacteroidetes*. *Proteobacteria* plays an essential role in promoting plant growth by nutrient acquisition and suppression of disease and can use a wide range of carbon substances derived from roots (root exudation and decomposing residues) (Philippot et al. 2013b; Xiong et al. 2015). The phylum *Planctomycetes* contains microbes that are antagonistic to the causal agent of wilt diseases in different crops (Chen et al. 2018). Similarly, *Firmicutes* and *Bacteroidetes* were involved in suppressing the growth of *Rhizoctonia Solani* (Ali et al. 2019b; Li and Wu 2018). *Actinobacteria* can produce a wide range of antibiotics that inhibit the growth and development of a diverse range of plant pathogens (Aziz et al. 2021; Li and Wu 2018).

Leafy vegetables suppress the growth of several phyla, such as *Acidobacteria*, *Chloroflexi*, *Gemmatimonadetes*, and *Nitrospirae*. *Acidobacteria* is thought to be slow-growing bacteria (Fierer et al. 2007), and we have observed a low abundance of *Acidobacteria* in the leafy vegetable planting system. It is more likely due to *Acidobacteria* being more adapted to the nutrient-limited soil environment (Fierer et al. 2012). *Nitrospirae* plays a key role in the ammonia and nitrite oxidation process (Norton 2008; Shaw et al. 2006). Reduction in the abundance of *Nitrospirae* in the leafy vegetable cropping system can reduce the nitrification process and, consequently, enhance the availability of ammonia nitrogen and reduce the loss of nitrogen through leaching and denitrification (Norton and Ouyang 2019). A similar pattern was observed in our previous study, where winter leafy vegetables decrease the abundance of *Nitrospirae* by increasing nitrogen availability (Ali et al. 2019b). *Gemmatimonadetes* abundance was higher in FE plots as compared to rotation treatments. This is another important finding of our study because *Gemmatimonadete* encompasses denitrifiers capable of converting soil  $\text{NO}_3\text{-N}$  into  $\text{N}_2$  or  $\text{N}_2\text{O}$ , resulting in nitrogen loss and declining soil fertility and crop yield (Yang et al. 2018).

The CR system increased several beneficial microbes at the genus level and suppressed



pathogenic bacteria. For example, *Xanthomonadales* have been previously documented as a disease-causing pathogen and cause yield reduction to more than 400 agricultural plants (Okubara and Bonsall 2008). CR promoted the growth of certain beneficial microbes, which had an antagonistic impact on soil-borne pathogens and revealed an increase in plant growth by releasing active secondary metabolites in soil, e.g., *Bacillus* is reported to promote plant growth with the ability to fight against soilborne pathogens by secreting active secondary metabolites (Egamberdieva 2016). *Pseudarthrobacter* has the potential to degrade the concentration of 4-chlorophenol and may be helpful in the bioremediation process (Jansson 2000). *Lysobacter* is another plant growth-promoting bacteria found in higher abundance in the CR system, capable of suppressing the phytopathogens by releasing various antimicrobial compounds and extracellular enzymes (Gómez Expósito et al. 2015).

The literature has widely discussed that *Fusarium oxysporum* is one of the key pathogenic fungi which causes Fusarium wilt in eggplant (Safikhani et al. 2013). Reduction in the abundance of *Fusarium* exhibited that leafy vegetable as a cover crop is an effective management practice to prevent soilborne plant diseases of eggplant. Besides, leafy vegetables excrete different kinds of allelochemicals, especially NCC (Yeo et al. 2021). The Brassicaceae family that produces secondary metabolites such as glucosinolates and their hydrolysis products can inhibit soilborne pathogens, including *Fusarium oxysporum*, a dominant fungal plant pathogen, and cause many plant diseases (Liu et al. 2019). *Botrytis* is another important eggplant pathogenic fungus that causes gray mold disease in eggplant, and it can reduce 20–30% of eggplant production (D.C. Mosou). Rotation with winter leafy vegetables reduced the botrytis. Our results align with the previous study by Lyu et al. (2020) that crop rotation with winter leafy vegetables efficiently reduced botrytis.

Cover crops used as a crop rotation have significant positive effects on soil microbial community and diversity structure and increased beneficial microbes (Liu et al. 2021; Lyu et al. 2020). CR increases the relative abundance of some beneficial fungal genera, suppressing the different diseases in vegetables such as *Mortierella* and *Acremonium*. *Mortierella* inhibits many pathogens by producing antibiotics (Melo et al. 2014). Similarly, *Acremonium* also acts as a

biocontrol against many diseases by producing lytic enzymes and antimicrobial compounds (Anisha and Radhakrishnan 2015). The abundance of *Hypocreales* in NCC rotation was reduced as compared to FE cropping system in both years. *Hypocreales* have been identified to play a vital role in denitrification and reduced nitrogen losses (Risgaard-Petersen et al. 2006). Thus, a reduction in the abundance of *Hypocreales* under NCC rotation suggests the importance of NCC in a crop rotation. The CR with winter leafy vegetables used in this study effectively suppresses soilborne disease pathogens caused by continuous monocropping of eggplant.

#### Relationships between soil edaphic factors and soil bacteria and fungi

Cover crops used as a crop rotation effectively protect vegetable production and soil sustainability by altering the soil microenvironment and regulating soil fertility by improving soil physio-chemical properties (Blanco-Canqui et al. 2015). Enormous studies have exhibited that environmental factors shape microbial community structure and community (Ali et al. 2019b; Ghani et al. 2019b; Lyu et al. 2020; Pathan et al. 2020). The fungal genus *Mortierella* had a positive correlation with soil biochemical properties such as urease, Invertase, phosphatase,  $\beta$ -glucosidase, AN, MBN, MBC, SOC, yield, and pH. *Mortierella* is a slow-growing K-strategist fungi known to assist crops and mycorrhizal fungi in phosphorus acquisition (Li et al. 2018; Ozimek and Hanaka 2020). Further, it has the functional capacity to decompose recalcitrant substances and also harbors genes associated with the degradation of toxic substances, thereby improving soil health (Li et al. 2017; Nakagawa et al. 2006). Soil enzymatic activity, SOC, AN, AK, MBC, and MBN were the main factors influencing soil bacterial community structure. Some taxa such as *Solirubrobacter* and *Nocradioides* had a positive while *Bacillus*, *Acidimicorbiales*, *Gemmatimonadaceae*, etc., had a negative correlation with these parameters. Our results are in line with the previous finding, which shows that influence of soil quality parameters on composition of the bacterial population is highly dispartates and fluctuates with taxa to taxa (Fernández-Calviño and Bååth 2016; Lyu et al. 2020; Rousk et al. 2010; Sánchez-Marañón et al. 2017).

## Conclusion

Within the context of the transition of agricultural practices towards sustainable vegetable cultivation, our findings indicate that different leafy vegetable species used as a cover crop in crop rotations could be successfully used to minimize external inputs without reducing yield by improving degraded soil under the plastic tunnel. Conclusively, cover crops, winter leafy vegetables including WO, CL, NCC, and LT crop improved physiochemical properties and bacterial and fungal community and diversity structure by increasing soil beneficial microbes and suppressing soilborne pathogen and hence improved eggplant yield. Moreover, the impact on soil microbial community was more evident with increasing cover crop rotation time, i.e., more than one year. Among different leafy vegetable species studied in this experiment, NCC and CL were more effective in alleviating the negative impact of continuous cropping.

**Acknowledgments** This research was supported by the projects of Shaanxi Provincial Sci-Tech Innovation Plan (Grant No. 2016KTCL02-01), and the National Natural Science Foundation (Grant No. 31772293) of P.R. China.

**Authors' contribution** Muhammad Imran Ghani: Experimentation, Investigation, Data analysis, Methodology, Writing- original draft, Writing - review & editing. Ahmad Ali: Soil sampling, Data collection, Data analysis. Muhammad Jawaad Atif: Methodology, Software, Data curation.: Shamina Imran Pathan, Visualization, Data interpretation, Writing - review & editing. Giacomo Pietramellara: Visualization, Writing - review & editing. Muhammad Ali, Bakht Amin: Soil sampling, Data collection, Data analysis. Zhihui Cheng: Conceptualization, Supervision, Writing - review & editing.

## Declarations

**Competing interests** The authors declare no conflict of interest.

## References

- Adams RI, Miletto M, Taylor JW, Bruns TD (2013) Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J* 7:1262–1273
- Ali A, Ghani MI, Ding H, Fan Y, Cheng Z, Iqbal M (2019a) Co-amended synergistic interactions between arbuscular mycorrhizal fungi and the organic substrate-induced cucumber yield and fruit quality associated with the regulation of the am-fungal community structure under anthropogenic cultivated soil. *Int J Mol Sci* 20:1539
- Ali A, Imran Ghani M, Li Y, Ding H, Meng H, Cheng Z (2019b) Hiseq base molecular characterization of soil microbial community, diversity structure, and predictive functional profiling in continuous cucumber planted soil affected by diverse cropping systems in an intensive greenhouse region of northern China. *Int J Mol Sci* 20:2619
- Ali A, Ghani MI, Elrys AS, Ding H, Iqbal M, Cheng Z, Cai Z (2021) Different cropping systems regulate the metabolic capabilities and potential ecological functions altered by soil microbiome structure in the plastic shed mono-cropped cucumber rhizosphere. *Agric Ecosyst Environ* 318:107486
- Alvey S, Yang C-H, Bürkert A, Crowley D (2003) Cereal/legume rotation effects on rhizosphere bacterial community structure in West African soils. *Biol Fertil Soils* 37:73–82
- Anisha C, Radhakrishnan E (2015) Gliotoxin-producing endophytic *Acremonium* sp. from *Zingiber officinale* found antagonistic to soft rot pathogen *Pythium myriophyllum*. *Appl Biochem Biotechnol* 175:3458–3467
- Ashworth A, DeBruyn J, Allen F, Radosevich M, Owens P (2017) Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. *Soil Biol Biochem* 114:210–219
- Austin EE, Wickings K, McDaniel MD, Robertson GP, Grandy AS (2017) Cover crop root contributions to soil carbon in a no-till corn bioenergy cropping system. *GCB Bioenergy* 9:1252–1263
- Aziz U, Rehmani MS, Wang L, Luo X, Xian B, Wei S, Wang G, Shu K (2021) Toward a Molecular Understanding of Rhizosphere, Phyllosphere, and Spherosphere Interactions in Plant Growth and Stress Response. *Crit Rev Plant Sci* 40(6):479–500. <https://doi.org/10.1080/07352689.2022.2031728>
- Bissett A, Richardson AE, Baker G, Kirkegaard J, Thrall PH (2013) Bacterial community response to tillage and nutrient additions in a long-term wheat cropping experiment. *Soil Biol Biochem* 58:281–292
- Blanco-Canqui H, Shaver TM, Lindquist JL, Shapiro CA, Elmore RW, Francis CA, Hergert GW (2015) Cover crops and ecosystem services: insights from studies in temperate soils. *Agron J* 107:2449–2474
- Bottner P, Sallih Z, Billes G (1988) Root activity and carbon metabolism in soils. *Biol Fertil Soils* 7:71–78
- Brennan EB, Acosta-Martinez V (2017) Cover cropping frequency is the main driver of soil microbial changes during six years of organic vegetable production. *Soil Biol Biochem* 109:188–204
- Brussaard L, De Ruiter PC, Brown GG (2007) Soil biodiversity for agricultural sustainability. *Agric Ecosyst Environ* 121:233–244
- Chahal I, Van Eerd LL (2020) Cover crop and crop residue removal effects on temporal dynamics of soil carbon and nitrogen in a temperate, humid climate. *PLoS One* 15:e0235665
- Chamberlain LA, Bolton ML, Cox MS, Suen G, Conley SP, Ane J-M (2020) Crop rotation, but not cover crops, influenced soil bacterial community composition in a

- corn-soybean system in southern Wisconsin. *Appl Soil Ecol* 154:103603
- Chang C-L, Fu X-P, Zhou X-G, Guo M-Y, Wu F-Z (2017) Effects of seven different companion plants on cucumber productivity, soil chemical characteristics and *Pseudomonas* community. *J Integr Agric* 16:2206–2214
- Chen C, Liu C-H, Cai J, Zhang W, Qi W-L, Wang Z, Liu Z-B, Yang Y (2018) Broad-spectrum antimicrobial activity, chemical composition and mechanism of action of garlic (*Allium sativum*) extracts. *Food Control* 86:117–125
- Coombs C, Lauzon JD, Deen B, Van Eerd LL (2017) Legume cover crop management on nitrogen dynamics and yield in grain corn systems. *Field Crop Res* 201:75–85
- Cotrufo MF, Wallenstein MD, Boot CM, Deneff K, Paul E (2013) The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Glob Chang Biol* 19:988–995
- Daryanto S, Fu B, Wang L, Jacinthe P-A, Zhao W (2018) Quantitative synthesis on the ecosystem services of cover crops. *Earth Sci Rev* 185:357–373
- De Long JR, Fry EL, Veen G, Kardol P (2019) Why are plant–soil feedbacks so unpredictable, and what to do about it? *Funct Ecol* 33:118–128
- Ding H, Ali A, Cheng Z (2018) Dynamics of a soil fungal community in a three-year green garlic/cucumber crop rotation system in Northwest China. *Sustainability* 10:1391
- Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 10(10):996–998. <https://doi.org/10.1038/nmeth.2604>
- Egamberdieva D (2016) *Bacillus* spp.: a potential plant growth stimulator and biocontrol agent under hostile environmental conditions. In: Islam M, Rahman M, Pandey P, Jha C, Aeron A (eds) *Bacilli and Agrobiotechnology*. Springer, Cham, pp 91–111
- Eivazi F, Tabatabai M (1988) Glucosidases and galactosidases in soils. *Soil Biol Biochem* 20:601–606
- Fernández-Calviño D, Bååth E (2016) Interaction between pH and Cu toxicity on fungal and bacterial performance in soil. *Soil Biol Biochem* 96:20–29
- Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. *Ecology* 88:1354–1364
- Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R (2012) Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J* 6:1007–1017
- Finney D, Buyer J, Kaye J (2017) Living cover crops have immediate impacts on soil microbial community structure and function. *J Soil Water Conserv* 72:361–373
- Frasier I, Noellemeyer E, Figuerola E, Erijman L, Permingeat H, Quiroga A (2016) High quality residues from cover crops favor changes in microbial community and enhance C and N sequestration. *Glob Ecol Conserv* 6:242–256
- Fu H, Zhang G, Zhang F, Sun Z, Geng G, Li T (2017) Effects of continuous tomato monoculture on soil microbial properties and enzyme activities in a solar greenhouse. *Sustainability* 9:317
- Gale W, Cambardella C (2000) Carbon dynamics of surface residue—and root-derived organic matter under simulated no-till. *Soil Sci Soc Am J* 64:190–195
- Garbeva P, Van Veen J, Van Elsas J (2004) Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. *Annu Rev Phytopathol* 42:243–270
- Ghani MI, Ali A, Atif MJ, Ali M, Amin B, Anees M, Khurshid H, Cheng Z (2019b) Changes in the soil microbiome in eggplant monoculture revealed by high-throughput Illumina MiSeq sequencing as influenced by raw garlic stalk amendment. *Int J Mol Sci* 20:2125
- Ghani M, Ali A, Atif M, Ali M, Amin B, Anees M, Cheng Z (2019a) Soil Amendment with Raw Garlic Stalk: A Novel Strategy to Stimulate Growth and the Antioxidative Defense System in Monocropped Eggplant in the North of China. *Agronomy* 9(2):89. <https://doi.org/10.3390/agronomy9020089>
- Ghani MI, Ali A, Atif MJ, Ali M, Amin B, Cheng Z (2022) Arbuscular Mycorrhizal Fungi and Dry Raw Garlic Stalk Amendment Alleviate Continuous Monocropping Growth and Photosynthetic Declines in Eggplant by Bolstering Its Antioxidant System and Accumulation of Osmolytes and Secondary Metabolites. *Front Plant Sci* 13:849521. <https://doi.org/10.3389/fpls.2022.849521>
- Gómez Expósito R, Postma J, Raaijmakers JM, De Bruijn I (2015) Diversity and activity of *Lysobacter* species from disease suppressive soils. *Front Microbiol* 6:1243
- Guo R, Li X, Christie P, Chen Q, Jiang R, Zhang F (2008) Influence of root zone nitrogen management and a summer catch crop on cucumber yield and soil mineral nitrogen dynamics in intensive production systems. *Plant Soil* 313:55–70
- Hannula SE, Heinen R, Huberty M, Steinauer K, De Long JR, Jongen R, Bezemer TM (2021) Persistence of plant-mediated microbial soil legacy effects in soil and inside roots. *Nat Commun* 12(1):5686. <https://doi.org/10.1038/s41467-021-25971-z>
- Heinen R, van der Sluijs M, Biere A, Harvey JA, Bezemer TM (2018) Plant community composition but not plant traits determine the outcome of soil legacy effects on plants and insects. *J Ecol* 106:1217–1229
- Hu W, Zhang Y, Huang B, Teng Y (2017) Soil environmental quality in greenhouse vegetable production systems in eastern China: current status and management strategies. *Chemosphere* 170:183–195
- Jansson JK (2000) *Arthrobacter chlorophenolicus* sp. nov., a new species capable of degrading high concentrations of 4-chlorophenol. *Int J Syst Evol Microbiol* 50:2083–2092
- Jenkinson DS, Ladd J (1981) Microbial biomass in soil: measurement and turnover. *Soil Biochem* 5:415–471
- Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant Soil* 321:5–33
- Kanders MJ, Berendonk C, Fritz C, Watson C, Wichern F (2017) Catch crops store more nitrogen below-ground when considering Rhizodeposits. *Plant Soil* 417:287–299
- Knudsen D, Peterson G, Pratt P (1983) Lithium, sodium, and potassium. *Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties* 9: 225–246
- Kong AY, Six J (2010) Tracing root vs. residue carbon into soils from conventional and alternative cropping systems. *Soil Sci Soc Am J* 74:1201–1210

- Kong AY, Six J (2012) Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems. *Plant Soil* 356:315–330
- Lai C-Y, Wen L-L, Zhang Y, Luo S-S, Wang Q-Y, Luo Y-H, Chen R, Yang X, Rittmann BE, Zhao H-P (2016) Auto-trophic antimonate bio-reduction using hydrogen as the electron donor. *Water Res* 88:467–474
- Lehman RM, Taheri WI, Osborne SL, Buyer JS, Douds DD Jr (2012) Fall cover cropping can increase arbuscular mycorrhizae in soils supporting intensive agricultural production. *Appl Soil Ecol* 61:300–304
- Lehman RM, Cambardella CA, Stott DE, Acosta-Martinez V, Manter DK, Buyer JS, Maul JE, Smith JL, Collins HP, Halvorson JJ (2015) Understanding and enhancing soil biological health: the solution for reversing soil degradation. *Sustainability* 7:988–1027
- Li S, Wu F (2018) Diversity and co-occurrence patterns of soil bacterial and fungal communities in seven intercropping systems. *Front Microbiol* 9:1521
- Li W, Cheng Z, Meng H, Zhou J, Liang J, Liu X (2012) Effect of rotating different vegetables on micro-biomass and enzyme in tomato continuous cropped substrate and afterculture tomato under plastic tunnel cultivation. *Acta Horticult Sinica* 39:73–80
- Li X-g, Ding C-f, Zhang T-l, Wang X-x (2014) Fungal pathogen accumulation at the expense of plant-beneficial fungi as a consequence of consecutive peanut monoculturing. *Soil Biol Biochem* 72:11–18
- Li T, Liu T, Zheng C, Kang C, Yang Z, Yao X, Song F, Zhang R, Wang X, Xu N (2017) Changes in soil bacterial community structure as a result of incorporation of Brassica plants compared with continuous planting eggplant and chemical disinfection in greenhouses. *PLoS One* 12:e0173923
- Li F, Chen L, Redmile-Gordon M, Zhang J, Zhang C, Ning Q, Li W (2018) *Mortierella elongata*'s roles in organic agriculture and crop growth promotion in a mineral soil. *Land Degrad Dev* 29:1642–1651
- Lienhard P, Terrat S, Prévost-Bouré NC, Nowak V, Régnier T, Sayphoummie S, Panyasiri K, Tivet F, Mathieu O, Levêque J (2014) Pyrosequencing evidences the impact of cropping on soil bacterial and fungal diversity in Laos tropical grassland. *Agron Sustain Dev* 34:525–533
- Liu M, Wu F, Wang S, Lu Y, Chen X, Wang Y, Gu A, Zhao J, Shen S (2019) Comparative transcriptome analysis reveals defense responses against soft rot in Chinese cabbage. *Horticult Res* 6:1–18
- Liu X, Hannula SE, Li X, Hundscheid MP, Klein Gunnewiek PJ, Clocchiatti A, Ding W, de Boer W (2021) Decomposing cover crops modify root-associated microbiome composition and disease tolerance of cash crop seedlings. *Soil Biol Biochem* 160:108343
- Logsdon S, Kaspar TC, Meek DW, Prueger JH (2002) Nitrate leaching as influenced by cover crops in large soil monoliths. *Agron J* 94:807–814
- López-Bellido L, López-Bellido RJ, Redondo R, Benítez J (2006) Faba bean nitrogen fixation in a wheat-based rotation under rainfed Mediterranean conditions: effect of tillage system. *Field Crop Res* 98:253–260
- Lyu J, Jin L, Jin N, Xie J, Xiao X, Hu L, Tang Z, Wu Y, Niu L, Yu J (2020) Effects of different vegetable rotations on fungal community structure in continuous tomato cropping matrix in greenhouse. *Front Microbiol* 11:829
- McDaniel M, Tiemann L, Grandy A (2014) Does agricultural crop diversity enhance soil microbial biomass and organic matter dynamics? A meta-analysis. *Ecol Appl* 24:560–570
- Melo IS, Santos SN, Rosa LH, Parma MM, Silva LJ, Queiroz SC, Pellizari VH (2014) Isolation and biological activities of an endophytic *Mortierella alpina* strain from the Antarctic moss *Schistidium antarctici*. *Extremophiles* 18:15–23
- Mendes LW, Tsai SM, Navarrete AA, De Hollander M, van Veen JA, Kuramae EE (2015) Soil-borne microbiome: linking diversity to function. *Microb Ecol* 70:255–265
- Meng P-P, Liu X, Qiu H-Z, Zhang W-M, Zhang C-H, Wang D, Zhang J-L, Shen Q-R (2012) Fungal population structure and its biological effect in rhizosphere soil of continuously cropped potato. *Ying yong sheng tai xue bao* = *J Appl Ecol* 23:3079–3086
- Motisi N, Montfort F, Doré T, Romillac N, Lucas P (2009) Duration of control of two soilborne pathogens following incorporation of above-and below-ground residues of *Brassica juncea* into soil. *Plant Pathol* 58:470–478
- Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. *Anal Chim Acta* 27:31–36
- Nakagawa A, Osawa S, Hirata T, Yamagishi Y, Hosoda J, Horikoshi T (2006) 2, 4-Dichlorophenol degradation by the soil fungus *Mortierella* sp. *Biosci Biotechnol Biochem* 70:525–527
- Nelson D, Martin J, Ervin J (1979) Decomposition of microbial cells and components in soil and their stabilization through complexing with model humic acid-type phenolic polymers. *Soil Sci Soc Am J* 43:84–88
- Norton JM (2008) Nitrification in agricultural soils. *Nitro Agric Syst* 49:173–199
- Norton J, Ouyang Y (2019) Controls and adaptive management of nitrification in agricultural soils. *Front Microbiol* 10:1931
- Okubara PA, Bonsall RF (2008) Accumulation of *Pseudomonas*-derived 2, 4-diacetylphloroglucinol on wheat seedling roots is influenced by host cultivar. *Biol Control* 46:322–331
- Ozimek E, Hanaka A (2020) *Mortierella* species as the plant growth-promoting Fungi present in the agricultural soils. *Agriculture* 11:1–1
- Pathan SI, Scibetta S, Grassi C, Pietramellara G, Orlandini S, Ceccherini MT, Napoli M (2020) Response of soil bacterial community to application of organic and inorganic phosphate based fertilizers under *Vicia faba* L. cultivation at two different Phenological stages. *Sustainability* 12:9706
- Pervaiz ZH, Iqbal J, Zhang Q, Chen D, Wei H, Saleem M (2020) Continuous cropping alters multiple biotic and abiotic indicators of soil health. *Soil Syst* 4:59
- Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013a) Going back to the roots: the microbial ecology of the rhizosphere. *Nat Rev Microbiol* 11:789–799

- Philippot L, Spor A, Hénault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron P-A (2013b) Loss in microbial diversity affects nitrogen cycling in soil. *ISME J* 7:1609–1619
- Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moëgne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil* 321:341–361
- Risgaard-Petersen N, Langezaal AM, Ingvarðsen S, Schmid MC, Jetten MS, den Camp HJO, Derksen JW, Pina-Ochoa E, Eriksson SP, Nielsen LP (2006) Evidence for complete denitrification in a benthic foraminifer. *Nature* 443:93–96
- Rousk J, Brookes PC, Bååth E (2010) Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biol Biochem* 42:926–934
- Safikhani N, Morid B, Zamanizadeh HR (2013) First report of fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp. *melongenae* in Iran. *New Disease Reports* 28
- Sánchez-Marañón M, Miralles I, Aguirre-Garrido JF, Anguita-Maeso M, Millán V, Ortega R, García-Salcedo JA, Martínez-Abarca F, Soriano M (2017) Changes in the soil bacterial community along a pedogenic gradient. *Sci Rep* 7:1–11
- Scarascia-Mugnozza G, Sica C, Russo G (2011) Plastic materials in European agriculture: actual use and perspectives. *J Agric Eng* 42:15–28
- Schmidt MW, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DA (2011) Persistence of soil organic matter as an ecosystem property. *Nature* 478:49–56
- Shaw LJ, Nicol GW, Smith Z, Fear J, Prosser JI, Baggs EM (2006) *Nitrosospora* spp. can produce nitrous oxide via a nitrifier denitrification pathway. *Environ Microbiol* 8:214–222
- Shi R (1996) *Agricultural chemistry analyses of soils*, 2nd edn. China Agricultural Press, Beijing, pp 37–39
- Shipley PR, Messinger J, Decker A (1992) Conserving residual corn fertilizer nitrogen with winter cover crops. *Agron J* 84:869–876
- Sokol NW, Bradford MA (2019) Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nat Geosci* 12:46–53
- Strickland MS, McCulley RL, Nelson JA, Bradford MA (2015) Compositional differences in simulated root exudates elicit a limited functional and compositional response in soil microbial communities. *Front Microbiol* 6:817
- Strock JS, Porter PM, Russelle M (2004) Cover cropping to reduce nitrate loss through subsurface drainage in the northern US Corn Belt. *J Environ Qual* 33:1010–1016
- Tabatabai M (1994) Soil enzymes. *Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties* 5: 775–833
- Tabatabai M, Bremner J (1972) Assay of urease activity in soils. *Soil Biol Biochem* 4:479–487
- Thapa VR, Ghimire R, Acosta-Martínez V, Marsalis MA, Schipanski ME (2021) Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems. *Appl Soil Ecol* 157:103735
- Tian Y, Liu J, Zhang X, Gao L (2010) Effects of summer catch crop, residue management, soil temperature and water on the succeeding cucumber rhizosphere nitrogen mineralization in intensive production systems. *Nutr Cycl Agroecosyst* 88:429–446
- Tian Y, Zhang X, Liu J, Gao L (2011) Effects of summer cover crop and residue management on cucumber growth in intensive Chinese production systems: soil nutrients, microbial properties and nematodes. *Plant Soil* 339:299–315
- Van Der Heijden MG, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* 11:296–310
- Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703–707
- Venter ZS, Jacobs K, Hawkins H-J (2016) The impact of crop rotation on soil microbial diversity: a meta-analysis. *Pedobiologia* 59:215–223
- Wang M, Wu C, Cheng Z, Meng H, Zhang M, Zhang H (2014) Soil chemical property changes in eggplant/garlic relay intercropping systems under continuous cropping. *PLoS One* 9:e111040
- Wang M, Wu C, Cheng Z, Meng H (2015) Growth and physiological changes in continuously cropped eggplant (*Solanum melongena* L.) upon relay intercropping with garlic (*Allium sativum* L.). *Front Plant Sci* 6:262
- Wang W, Han L, Zhang X (2020) Winter cover crops effects on soil microbial characteristics in sandy areas of Northern Shaanxi, China. *Revista Brasileira de Ciência do Solo* 44:e0190173. <https://doi.org/10.36783/18069657rbcs20190173>
- White KE, Brennan EB, Cavigelli MA, Smith RF (2022) Winter cover crops increased nitrogen availability and efficient use during eight years of intensive organic vegetable production. *PLoS One* 17:e0267757
- Wortman SE, Drijber RA, Francis CA, Lindquist JL (2013) Arable weeds, cover crops, and tillage drive soil microbial community composition in organic cropping systems. *Appl Soil Ecol* 72:232–241
- Xiao H, Fan X, Sun H, Yu M, Shi W, Singh BP, Wang H (2021) The benefit of leafy vegetable as catch crop to mitigate N and P leaching losses in intensive plastic-shed production system. *J Soils Sediments* 21:2253–2261
- Xiong W, Zhao Q, Zhao J, Xun W, Li R, Zhang R, Wu H, Shen Q (2015) Different continuous cropping spans significantly affect microbial community membership and structure in a vanilla-grown soil as revealed by deep pyrosequencing. *Microb Ecol* 70:209–218
- Yang L, Tan L, Zhang F, Gale WJ, Cheng Z, Sang W (2018) Duration of continuous cropping with straw return affects the composition and structure of soil bacterial communities in cotton fields. *Can J Microbiol* 64:167–181
- Yeo HJ, Baek S-A, Sathasivam R, Kim JK, Park SU (2021) Metabolomic analysis reveals the interaction of primary and secondary metabolism in white, pale green, and green pak choi (*Brassica rapa* subsp. *chinensis*). *Appl Biol Chem* 64:1–16
- Zhang F, Li S, Xiao D, Zhao J, Wang R, Guo X, Wang S (2015) Progress in pest management by natural enemies in greenhouse vegetables in China. *Sci Agric Sin* 48:3463–3476

- Zhang Z, Sun D, Tang Y, Zhu R, Li X, Gruda N, Dong J, Duan Z (2021) Plastic shed soil salinity in China: Current status and next steps. *J Clean Prod* 296:126453. <https://doi.org/10.1016/j.jclepro.2021.126453>
- Zhou X, Liu J, Wu F (2017) Soil microbial communities in cucumber monoculture and rotation systems and their feedback effects on cucumber seedling growth. *Plant Soil* 415:507–520
- Zhu Y, Fox R (2003) Corn–soybean rotation effects on nitrate leaching. *Agron J* 95:1028–1033

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.