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REGULAR ARTICLE

Changes in physicochemical properties, enzymatic activities, and the microbial community of soil significantly influence the continuous cropping of *Panax quinquefolius* L. (American ginseng)

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Abstract
Aims In the production of the natural medicinal plant American ginseng, replantation typically fails due to continuous cropping obstacles. However, the cause is still not clear and needs more research.
Methods Soil samples were collected from (a) maize fields where American ginseng had never been planted, (b) fields where American ginseng had just been harvested, and (c) fields where maize had been planted for 2, 4 and 6 years respectively after American ginseng. We investigated the physicochemical properties, the enzymatic activities, and the soil microbial community structure and composition of the samples.
Results We found that the content of soil salt, NH_4^+-N, and NO_3^--N increased significantly in samples associated with the production of American ginseng, whereas the soil pH, carbon-to-nitrogen ratio, alkaline phosphatase, and cellulase activity all significantly decreased and gradually recovered to the pre-planting level. Moreover, the bacterial diversity decreased, while fungal diversity and richness increased; fungal richness continued to increase in farmlands replanted maize. The relative abundance of some microbial communities was changed significantly and was gradually restored with a longer time to replant maize. Pearson's correlation analysis shown that significantly changed microbial communities were significantly associated with changes in soil pH, soil salt and nitrogen content, alkaline phosphatase, and cellulase activity.
Conclusions Changes in soil pH, soil salt and nitrogen content caused changes in microbial community structure and composition, as well as cellulase and alkaline phosphatase activity. These changes may cause the continuous cropping obstacles of American ginseng and may be improved by planting maize.

Keywords American ginseng · Continuous cropping obstacle · Soil microbial communities · Enzymatic activity

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Introduction

American ginseng (*Panax quinquefolius* L.) is a remarkable natural herbal medicine. It is believed to possess eutherapeutic effects that enhance central nervous system function, protect the cardiovascular system, and improve immunity, due to its anti-inflammatory and antioxidant properties (Kitts et al. 2000; Wang et al. 2015a, b; Wu et al. 2016; Yu et al. 2014). The plant is also employed in the treatment of diabetes (Sen et al.

2012). As an important herbal medicine, the global American ginseng market reached US$ 85 million in 2018, and it is expected that the market will reach 7,033 tons in 2019~2024 (Zhang 2019). China is the third largest production country for American ginseng and produced 3,600 tons of American ginseng in 2016 (Zhang et al. 2020). However, an important issue in its production is continuous cropping obstacle (He et al. 2009). This usually causes the seedling survival rate to drop below 30 % in continuous cropping (Jian et al. 2008). Therefore, its planting mode is typically cultivated continuously in 4-year cycles and improved the soil condition by planting maize after harvesting American ginseng in China.

Continuous cropping obstacle is generally believed to be caused by numerous biotic and abiotic factors, such as the deterioration of soil physicochemical properties (Kaur and Singh 2014; Reeves 1997; Zydlik and Zydlik 2013), autotoxicity of plants (Van Wyk et al. 2017; Zhang et al. 2007; Zhang and Lin 2009), and changes in the diversity and composition of microbial communities (Buyer et al. 2010; Dong et al. 2017; Ma et al. 2010). Soil microbial communities typically play a significant role in agricultural production, as they maintain soil health and quality (Jangid et al. 2008; Van Wyk et al. 2017). Considerable differences can exist in the abiotic characteristics, microbial abundances, rates of microbial activity, and microbial community composition within even extremely close soil environments that share the same geographies (Fierer 2017). Some studies have reported that the continuous cropping obstacle of multiple crops is critically influenced by changes in soil microbial community structure and composition (Gao et al. 2019; Tan et al. 2017). Furthermore, multiple studies have reported that the modification of resident soil microbial flora is related to changes in soil physicochemical properties, soil enzymatic activities (Becker et al. 2017; Schnecker et al. 2015; Zheng et al. 2019), planted species, and the type of soil environment (Berg and Smalla 2009; Dong et al. 2017).

The physicochemical properties of soil are essential factors that influence the composition of soil microbial communities. For example, soil pH has a greater influence on the composition of bacterial and archaeal communities than fungi (Blagodatskaya and Anderson 1998; Lauber et al. 2009; Zhalnina et al. 2015). Some studies have reported that changes in soil pH greatly impact the soil microbial community structure and contribute to continuous cropping obstacles (Gao et al. 2019; Tan et al. 2017). In addition, many other factors can directly or indirectly influence the spatial structure of soil microbial communities, such as soil moisture (Brockett et al. 2012; Drenovsky et al. 2004), nitrogen and phosphorus content (Bowles et al. 2014; Cederlund et al. 2014; Fierer 2017). Nitrogen is an important component of soil quality and is a necessary nutrient for plant growth and development (Jia et al. 2008; Zhang et al. 2015). Nitrogen content is also a critical factor that affects the composition and diversity of microbial communities (Koyama et al. 2014; Pan et al. 2014; Zheng et al. 2019). It is essential for the growth and metabolism of microbes (Wang et al. 2017) and serves as an indispensable energy and nutrient source for microorganisms. Nitrogen is taken up by most plant species in the form of nitrate (NO_3^-) or ammonium (NH_4^+) (Cramer and Lewis 1993; Horchani et al. 2010). As the oxidation of NH_4^+ to NO_3^- progresses and releases H^+, an increase in soil nitrate levels is typically accompanied by lower soil pH (Fageria and Baligar 2005). In most cases, changes in these soil physicochemical properties can also directly or indirectly influence the growth and development of plants and overall crop production. Furthermore, the salt content of soil is an essential factor that impacts the health of soil and plants, as well as the composition of microbial communities (Daliakopoulos et al. 2016; Shrivastava and Kumar 2015). A number of soil physicochemical processes and enzymatic activities might also be affected by the soil salt content (Pathak and Rao 1998; Wichern et al. 2006).

Numerous studies have investigated how soil physicochemical properties and microbial community composition influence enzyme activities pertaining to the regulation of extracellular enzyme levels. Fungi are thought to have a greater influence than bacteria on enzyme activities associated with the decomposition of organic matter, as well as in the generation of products that can provide labile resources for bacteria (Bowles et al. 2014; Schnecker et al. 2015). Extracellular enzymatic activity is a good indicator of carbon decomposition in soil (Sinsabaugh et al. 2005, 2014) and is thought to be closely related to C and N processes (Banerjee et al. 2016; Burns et al. 2013; Henry 2013). For example, soil microbes generate cellulases, a group of hydrolytic enzymes that decompose polysaccharides (Deng and Tabatabai 1994). Enzymes associated with microbial N-acquisition include b-1,4-N-acetyl-glucosaminidase, leucine amino peptidase, and urease (Eivazi and Tabatabai 1977; Hui et al. 2013; Tabatabai and Bremner 1972) and associated with P-acquisition include acidic phosphatase,

neutral phosphatase, and alkaline phosphatase (Hui et al. 2013). Previous studies have reported that the activity of alkaline phosphatase was related to continuous cropping obstacles (Meng et al. 2012; Wang et al. 2012).

Previous studies of continuous cropping obstacles of American Ginseng revealed that changes in the soil microbial communities during cultivation resulted in imbalanced microbial communities and a reduction in metabolic functionality (Qi et al. 2010; Ying et al. 2012). There is an obvious phenomenon of decreased bacterial diversity and increased fungal diversity in the soils of American ginseng cultivated in continuous crop versus traditional crop systems (Dong et al. 2017). However, the reasons for the changes in the structure and composition of the microbial community during continuous cropping of American Ginseng are not clear, and the theoretical basis for planting maize after American Ginseng can effectively improve this problem remains unclear. For this study, the physicochemical properties and enzymatic activities were compared among soils from (a) maize fields where American ginseng had never been planted, (b) fields where American ginseng had just been harvested, and (c) fields where maize had been planted for 2, 4 and 6 years respectively after American ginseng. The diversity and composition of bacterial and fungal communities were compared using high-throughput sequencing. The microecological causes of continuous cropping obstacles of American Ginseng were analyzed to provide scientific recommendations to improve the sustainable development of the medicinal plant industry. We hypothesized that (1) the planting of American ginseng changes some key soil physicochemical properties, which makes farmland unsuitable for the growth of American ginseng; (2) these changes influence related soil enzyme activities and microbial community structure and composition and leads to continuous cropping obstacles of American Ginseng; and (3) these changes are gradually restored to baseline conditions as the replanting maize time length increases after harvesting American Ginseng.

Materials and methods

Study sites and soil sample collection

Soil samples were collected in October 2015 in Liuba County, Shaanxi Province (106°54'32.50"E-106°54'41.32"E, 33°36'59.28"N-33°37'4.44"N), which is one of the main areas in China for American ginseng cultivation. Soil samples were randomly collected from (a) maize fields where American ginseng had never been planted (group A), (b) fields where American ginseng had just been harvested (group B), and (c) fields where maize had been planted for 2-years (group C), 4-years (group D), and 6-years (group E) respectively after American ginseng. In the process of experiment, organic fertilizer (2.0 $kg{\cdot}m^{-2}$) was applied as base fertilizer in October, and added 1.0 $kg{\cdot}m^{-2}$ in May of each year. In each field, samples were collected via a five-point sampling method to obtain representative samples. For each group, five soil samples were collected and combined to form a single sample, which was repeated three times, resulting in a total of 15 samples. The samples were aseptically extracted from the soil surface growth layer (0~20 cm depth), homogenized by sieving to 2 mm into a 50 ml centrifuge tube, and then immediately stored at 4 °C for further analysis, as well as at -80 °C, for sequencing.

Determination of soil physicochemical properties

An analysis of the physicochemical properties of the soil sample was performed at the Key Laboratory of Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry (NEL-ECCD) in Xi'an, China. The soil moisture (SM) was determined using a Sartorius MA 100 moisture test apparatus. Soils were air-dried to analyze the soil electric conductivity (SE), the soil salt content (SS), total soil organic carbon (SOC), total nitrogen (TN), total phosphorus, and available phosphorus (AP). Fresh soil samples were used to determine the soil pH and the content of soil ammonium nitrogen (NH_4^+-N, AN) and nitrate nitrogen (NO_3^--N, NN).

Following the homogenization of soil samples by sifting through a 1 mm sieve, the pH was measured via potentiometry in deionized water (soil: water = 1:5 (w:v)) using a pH meter. The SE was determined using a conductivity meter. According to the standard curve between the conductivity and concentration of the potassium chloride solution at room temperature, the following formula, C (g/L) = (S + 41.2653)/2120.76, was used to calculate the SS based on soil electric conductivity. The SOC was determined by the $K_2Cr_2O_7$ titrimetric method (Yeomans and Bremner 1988). The TN of the soil sample was determined by the $K_2Cr_2O_7$-H_2SO_4 digestion method (Flowers and Bremner 1991). The soil C/N ratio (CN) was calculated based on the

SOC and TN. The soil NN was determined by the phenol disulfonic acid method (Sahrawat and Prasad 1975). The AN was determined by a KCl extraction-indophenol blue colorimetry method (Wu et al. 2013). The TP was determined via the H_2SO_4-$HClO_4$ digestion method (Wei 2009). The AP was determined using the $NaHCO_3$ leaching molybdenum antimony colorimetric technique (Pu et al. 2014).

Determination of soil enzymatic activities

Urease activity (EC3.5.1.5) was assayed by colorimetric analysis of sodium phenate-sodium hypochlorite (Kandeler and Gerber 1988; Van Wyk et al. 2017). The activities of sucrase (EC3.2.1.26) and cellulase (EC3.2.1) were colorimetrically determined by DNS based on the decreasing sugar content (Li et al. 2016). Total phosphatase activity was colorimetrically estimated using disodium phenyl phosphate, which is a method based on the determination of the organic group content that is generated by the enzymatic reaction of disodium phenyl phosphate as the matrix. To facilitate determination, acid phosphatase (EC 3.1.3.2) with an acetate buffer (pH = 5.0 ~ 5.4), neutral phosphatase with a citrate buffer (pH = 7.0), and alkaline phosphatase (EC3.1.3.1) with a borate buffer (pH = 9 ~ 10) were used to ensure that the reaction occurred at an optimal pH (Tabatabai 1994). The dehydrogenase activity of the soil was detected through a TTC (triphenyl tetrazolium chloride) reduction method (Friedel et al. 1994). Catalase (EC1.11.1.6) activity was assayed by potassium permanganate titration (Sinha 1972).

Genomic DNA extraction and PCR amplification

The soil samples stored at -80 °C were used to extract DNA and to conduct PCR amplification and Illumina MiSeq sequencing; three replicates were used. The genomic DNA of the soil was extracted using a Soil DNA™ Kit (Sigma-Aldrich, Germany) according to the manufacturer's recommendations. The purity and concentration of the extracted DNA were determined using a spectrophotometer (Thermo Fischer Scientific, CA, USA), and integrity was determined using a 0.8 % agarose gel. Aliquots (40 μL) of high-quality DNA extracts were obtained for further analysis.

Subsequently, the bacterial V4 hypervariable regions of the 16S rRNA gene and fungal ITS1 region of the 18S rRNA gene were amplified via PCR from the microbial genome DNA using 520F (5'-AYTGGGYDTAAAGNG-3') and 802R (5'-TACNVGGGTATCTAATCC-3') primer pairs and ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'- GCTGCGTTCTTCATCGATGC-3') primer pairs, respectively.

PCR of the ITS1 region of the fungal 18 S rRNA was performed in triplicate in the same reaction mixtures as the V4 region; the A3 and D3 samples used 3 μL (20 ng/μL) of template, while the other samples used 2 μL (20 ng/μL) of template. The annealing temperature was 56 and was amplified using 28 cycles. The PCR product was excised from a 2.0 % agarose gel and purified using the QIAquick Gel Extraction Kit.

Illumina MiSeq sequencing and processing

Sequencing was performed using the Illumina MiSeq platform. Multiplexed DNA libraries were established by means of a DNA end-trimmed "A" base introduced at the 3' end, tagged adapter attachment and DNA fragment enrichment. Barcoded V4 and ITS1 amplicons were sequenced using the paired-end method by Illumina MiSeq with a six cycles index read. Following the statistical computations and analysis of the raw data, effective and high-quality sequences were screened for further analysis.

Next, the bacterial and fungal OTUs were identified using the same 97 % identity threshold, clustered, checked for chimeras, and assembled into incidence tables using UCHIME in mothur to remove the chimeric sequences and using UCLUST method in QIIME to cluster the high-quality sequences (Edgar 2010). A representative sequence was selected from each OTU using default parameters. Finally, the taxonomic assignment was made using the Greengenes Database and Unite database, respectively (Abarenkov et al. 2010; DeSantis et al. 2006). An OTU table was generated to record the abundance and taxonomy of each OTU in each sample. OTUs containing less than 0.001 % of total sequences across all samples were discarded. To minimize the difference in sequencing depth across samples, an averaged, rounded rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under 90 % of the minimum sequencing depth for further analysis.

Bioinformatics and statistical analysis

The rarefaction and rank abundance curves were established to evaluate the reliability and uniformity

coefficients of sequencing. Sequence data analyses were primarily performed using QIIME and R packages (v3.2.0). OTU-level alpha diversity indices, such as the Chao1 richness estimator, ACE metric (abundance-based coverage estimator), Shannon diversity index, and Simpson index, were calculated using the OTU table in QIIME. OTU-level ranked abundance curves were generated to compare the richness and evenness of OTUs between samples. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using UniFrac distance metrics (Lozupone and Knight 2005; Lozupone et al. 2007) and visualized via principal coordinate analysis (PCoA), nonmetric multidimensional scaling (NMDS), and unweighted pair-group method with arithmetic means (UPGMA) hierarchical clustering (Ramette 2007).

Differences in the UniFrac distances for pairwise comparisons between groups were determined using Student's t-test and the Monte Carlo permutation test with 1000 permutations and visualized through box-and-whisker plots. Principal component analysis (PCA) was also conducted based on genus-level compositional profiles (Ramette 2007). The taxonomy compositions and abundances were visualized using MEGAN (Huson et al. 2011) and GraPhlAn (Asnicar et al. 2015). Taxa abundances at the phylum, class, order, family, genus and species levels were statistically compared between samples or groups using Metastats (White et al. 2009), and visualized as violin plots.

Results

Soil physicochemical properties

The soil physicochemical properties are shown in Fig. 1. One-way ANOVA revealed that there were differences between different groups. Compared with group A, the SM and SOC of group B showed no significant change (Fig. 1a, e), while the SE, SS, TN, and NN were remarkably higher ($p < 0.01$). Furthermore, the SE, SS and NN came close to the levels of group A with a longer time to replant maize. The TN decreased remarkably ($p < 0.01$) in group C and then remarkably increased to the level of group B ($p < 0.01$) with the increase in replanting maize time; the TN of group E was significantly higher than that of group B ($p < 0.05$) (Fig. 1c, d, f, h). Simultaneously, the AN and AP exhibited a similar increasing trend between groups A and B, which recorded no significant difference, followed by a gradual decline observed in AN with a longer time to replant maize (Fig. 1g, j). Conversely, the soil pH was remarkably lower ($p < 0.01$) for group B than group A; soil pH was gradually regained in group C and remarkably higher ($p < 0.01$) in group D. Soil pH steadily increased with a longer time to replant maize, although group E never reached the pH level of group A (Fig. 1b). Similarly, the CN and TP were significantly lower ($p < 0.05$) across groups in group B compared to group A, continued to decline in group C, were gradually restored in group D, and returned to the level of group A in group E (Fig. 1k, i).

Soils enzymatic activity

The enzymatic activities of the soils of the five groups were compared and assayed, as shown in Fig. 2. When comparing groups A and B, we observed a remarkable difference in that the neutral phosphatase activity was significantly higher ($p < 0.01$) (Fig. 2a), whereas the alkaline phosphatase activity ($p < 0.01$) (Fig. 2b) and cellulase activity were considerably lower ($p < 0.01$) (Fig. 2d). No significant difference was found between groups regarding the total phosphatase activity (Fig. 2c), dehydrogenase activity (Fig. 2e), urease activity (Fig. 2f), acid phosphatase activity (Fig. 2g), sucrase activity (Fig. 2h), or catalase activity (Fig. 2i).

The alkaline phosphatase activity of group C was remarkably lower than that of group B ($p < 0.01$) and was considerably higher ($p < 0.01$) for group D. Although it tended to increase with replanting maize, the alkaline phosphatase activity of group E did not attain the level measured in soils that were never planted with American ginseng (Fig. 2b). In contrast, the cellulase activity of group C was significantly higher ($p < 0.05$) than that of group B. Higher cellulase activity was found to be directly related to replant maize time length; the cellulase activity of group E approached the levels of group A (Fig. 2d). In addition, the dehydrogenase activity exhibited a gradual increasing trend and was remarkably higher ($p < 0.01$) in group D and significantly higher ($p < 0.05$) in group E (Fig. 2e).

Sequencing and microbial community alpha diversity

A total of 634,124 and 1,718,305 valid chimera sequences from bacteria and fungi, respectively, were obtained by high-throughput sequencing analyses of

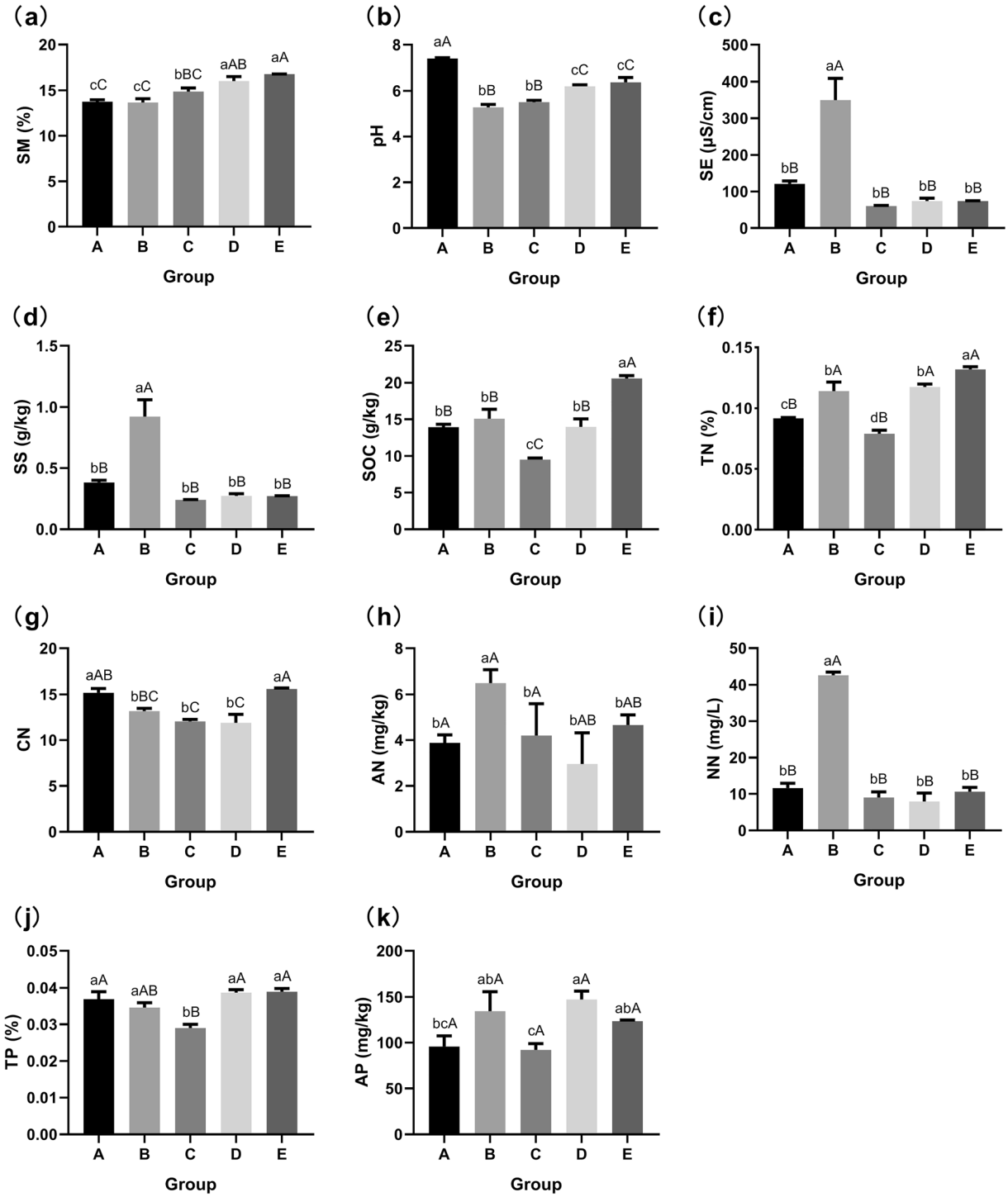


Fig. 1 Changes in soil physicochemical properties. Soil moisture (SM) (**a**), soil pH (**b**), soil electric conductivity (SE) (**c**), soil salt content (SS) (**d**), total soil organic carbon (SOC) (**e**), total nitrogen content (TN) (**f**), carbon-to-nitrogen ratio (CN) (**g**), ammonium nitrate content (NH_4^+-N, AN) (**h**), nitrate nitrogen content (NO_3^--N, NN) (**i**), total phosphorus content (**j**) and available phosphate (AP) (**k**) of both never planted American ginseng farmland (Group A), recently harvested American ginseng farmland (Group B), replanted maize for 2 years (Group C), 4 years (Group D) and 6 years (Group E) after planting American ginseng. Data are means ± standard deviation (n = 3). Different lowercase letters (a, b, c) indicate a significant difference at $p \leq 0.05$, whereas different uppercase letters (A, B, C) indicate a remarkable difference at $p \leq 0.01$

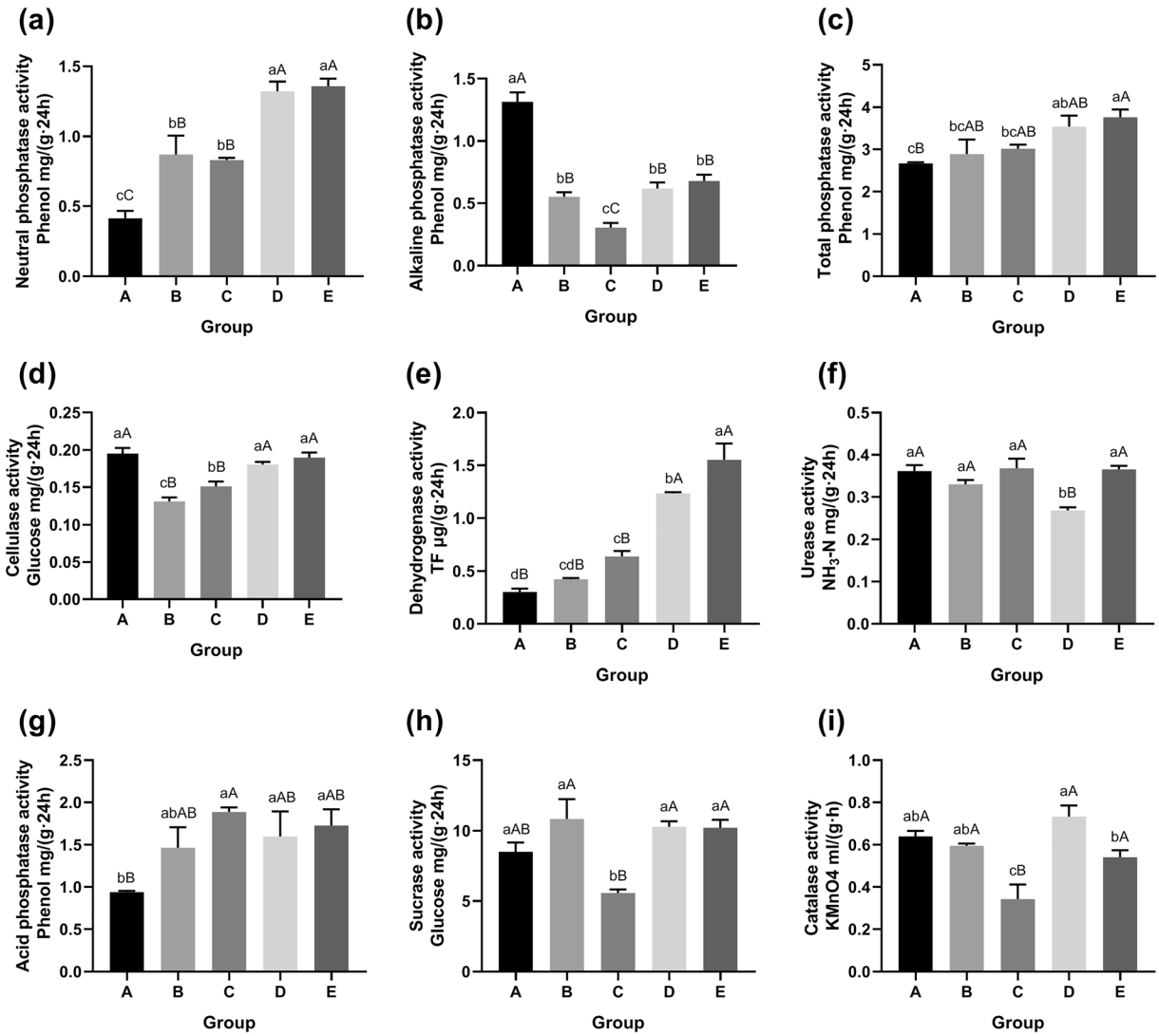


Fig. 2 Changes in soil enzymatic Activities. Neutral phosphatase (**a**), alkaline phosphatase (**b**), total phosphatase (**c**), cellulase (**d**), dehydrogenase (**e**), urease (**f**), acid phosphatase (**g**), sucrase (**h**) and catalase (**i**) of both never planted American ginseng farmland (Group A), recently harvested American ginseng farmland(Group B), replanted maize for 2 years (Group C), 4 years (Group D) and 6 years (Group E) after planting American ginseng. Different lowercase letters (a, b, c) indicate a significant difference at $p \leq 0.05$,

the V4 region of the 16 S rDNA gene and the ITS region of the 18 S rDNA, including 602,263 (94.98 %) and 1,504,849 (87.58 %) high-quality sequences, respectively, in the fifteen soil samples. The number of reads ranged from 22,704 to 47,955 and 80,576 to 123,250, respectively, which resulted in 9,544 and 1,160 OTUs (97 % cutoff) (Table S1). The lengths of the greatest number of reads were distributed across the ranges 224 ~ 226 and 223 ~ 281. The 31.24 % bacterial community OTUs and 52.16 % fungal community OTUs were shared in all five groups of soil samples, which accounted for the majority of the reads.

The OTU rarefaction and rank abundance curves indicated that a sufficient amount and quantity of sequencing data was obtained (Fig. S1), particularly for bacteria. Through the analyses of alpha diversity (Chao, Ace, Shannon, Simpson) (Table S2), we found that the levels of bacterial diversity in the soil tended to be lower after planting American ginseng and higher in the farmlands of replanting maize after American ginseng; however, the richness tended to be higher after planting American ginseng and lower in the farmlands of replanting maize (Fig. S2a, b, c, d). Nevertheless, levels of soil fungal diversity and richness both tended to be higher following

the planting of American ginseng and were persistently higher for the farmlands of replanting maize (Fig. S2e, f, g, h), although no significant difference was found.

The composition of bacterial and fungal communities

A total of 47 distinct bacterial phyla were detected across all fifteen samples. The most abundant sequences were affiliated with the phyla Bacteroidetes (14.2 %-28.2 % of total relative abundance) and Proteobacteria (12.7 %-23.7 %), followed by Planctomycetes (11.5 %-19.6 %), Acidobacteria (10.6 %-13.6 %), Actinobacteria (8.1 %-14.1 %), Gemmatimonadetes (4.1 %-6.5 %), Verrucomicrobia (3.8 %-6.3 %), and Chloroflexi (2.6 %-5.7 %) (Fig. 3a). Furthermore, Planctomycetia in the phylum Planctomycetes (10.3 %, 10.8 %, and 11.1 % in groups A, C, and E, respectively) was the dominant class, Alphaproteobacteria in the phylum Proteobacteria (10.4 %) was predominant in recently harvested farmland samples (group B), while Alphaproteobacteria represented 9.50 %, 6.90 %, 8.30 %, and 6.80 % in groups A, B, C, and E, respectively. A total of 6 distinct fungal phyla were detected across all fifteen samples and were dominated by the phylum Ascomycota (61.9 %-70.3 %), while the phyla Basidiomycota and Zygomycota represented only 7.6 %-8.6 % and 3.6 %-10.1 % reads, respectively (Fig. 3b). Furthermore, compared with group A, Sordariomycetes was decreased in group B and was gradually restored with a longer time to replant maize. Conversely, Leotiomycetes and Tremellomycetes were more abundant in group B than in group A and gradually decreased to the level of group A when the replanting maize time reached 6 years (Fig. 3d).

At the bacterial genus level, the relative abundances of *Chthoniobacter* ($p < 0.05$), *Opitutus* ($p < 0.01$), *Prosthecobacter* ($p < 0.05$), *Adhaeribacter* ($p < 0.05$), *Luteolibacter* ($p < 0.01$), *Agrobacterium* ($p < 0.05$), *Balneimonas* ($p < 0.05$), *Pontibacter* ($p < 0.05$), *Skermanella* ($p < 0.05$), and *Arthrobacter* ($p < 0.05$) in group B were significantly lower, representing 1.5 % of the sequences (Fig. 4a, S5). Furthermore, two genera (*Adhaeribacter* and *Luteolibacter*) were gradually restored with a longer time to replant maize. Correspondingly, group B were significantly higher in *DA101* ($p < 0.01$), *Mycobacterium* ($p < 0.05$), *Rhodoplanes* ($p < 0.01$),

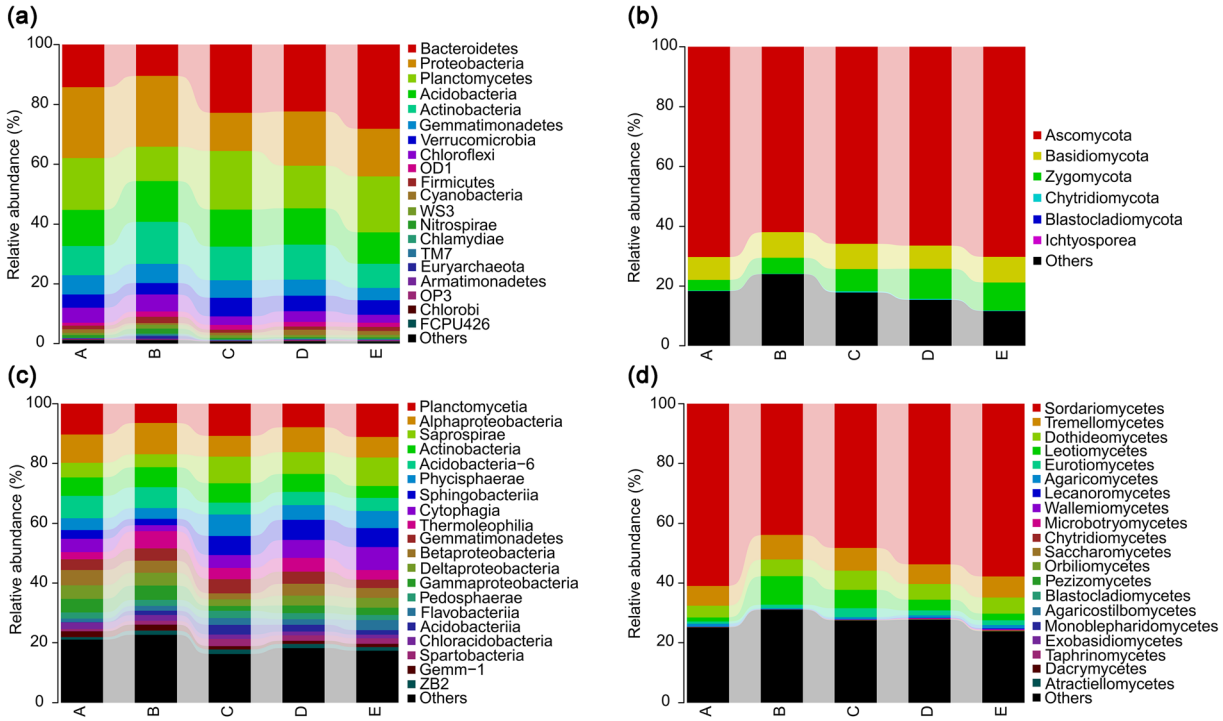


Fig. 3 Relative abundance of primary bacterial phyla (average relative abundance ≥ 0.5 %) (**a**), fungal phyla (average relative abundance ≥ 0.5 %) (**b**), bacterial class (average relative abundance ≥ 0.5 %) (**c**) and fungal class (average relative abundance ≥ 0.5 %) (**d**) present in different soil groups of bacterial and fungal communities

Pedobacter ($p < 0.05$), *Rhodanobacter* ($p < 0.01$) and *Bacillus* ($p < 0.05$). Except for *DA101*, which did not obviously change with a longer time to replant maize, other genera were gradually restored to the levels of group A (Fig. 4a, S6). At the fungal genus level, group B were significantly lower in *Myrothecium* ($p < 0.05$), *Volutella* ($p < 0.05$), *Wardomyces* ($p < 0.05$), *Thelebolus* ($p < 0.01$), *Leptosphaeria* ($p < 0.05$), *Passalora* ($p < 0.05$), *Truncatella* ($p < 0.01$), *Protomyces* ($p < 0.01$), *Toninia* ($p < 0.05$), *Rachicladosporium* ($p < 0.05$), *Hydnum* ($p < 0.01$), and *Marasmiellus* ($p < 0.01$) (Fig. 4b, S7), representing 1.5 % of the sequences. Furthermore, three genera (*Myrothecium*, *Hydnum*, and *Rachicladosporium*) were gradually restored as replanting maize time increased, whereas three genera (*Marasmiellus*, *Leptosphaeria*, and *Volutella*) did not obviously change with a longer time to replant maize. Additionally, three genera (*Thelebolus*, *Wardomyces*, and *Passalora*) were consistently lower in the farmlands of replanting maize. Only *Thelebolus* and *Wardomyces* were gradually restored in group E. Interestingly, *Toninia* was restored in group C but was again significantly lower in group D. Correspondingly, group B were significantly higher in *Retroconis* ($p < 0.05$), *Chaetomium* ($p < 0.05$), *Pseudeurotium* ($p < 0.05$), *Hypomyces* ($p < 0.01$), *Phialemonium* ($p < 0.01$), *Rhinocladiella* ($p < 0.01$), *Leptodiscella* ($p < 0.05$), *Torrubiella* ($p < 0.01$), *Monodictys* ($p < 0.05$), and *Cladosporium* ($p < 0.01$) and were gradually restored to the levels of group A with a longer time to replant maize. Although *Retroconis* and *Chaetomium* fluctuated by varying degrees during replanting maize periods, they also returned to group A levels (Fig. 4b, S8).

Bacterial and fungal Beta-diversity analysis and correlation with environmental parameters

The planting of American ginseng had a significant effect on the soil microbial community based on UniFrac distances. The Adonis analysis of the 16 S rRNA gene and ITS data using unweighted and weighted methods revealed that the bacterial and fungal community structures and compositions were significantly changed (Table 1). This phenomenon was also observed through PCoA and NMDS analyses (Fig. S3, S4). Significant variations between different groups were revealed by PCoA ordination for bacterial communities and fungi. PC1 (first principal component, 30.51 % contribution), PC2 (second principal component, 13.42 % contribution), and PC3 (third principal component, 11.39 % contribution) differentiated the bacterial communities (Fig. S3a, b, c); PC1 (first principal component, 17.88 % contribution), PC2 (second principal component, 12.98 % contribution), and PC3 (third principal component, 10.44 % contribution) differentiated the fungal communities (Fig. S3d, e, f). A similar phenomenon was observed via NMDS analysis (Fig. S4).

Redundancy analysis suggested that changes in soil chemistry played an important role in shaping the structure and composition of microbial communities. Changes in SM, NN, SS, SOC, and CN had a more obvious effect on the alteration of bacterial structures and compositions in different farmland soils; however, the changes in pH, TP, AN, AP, and TN had a relatively low impact (Fig. 5a). The changes in pH, SM, NN, SS, and CN had a more obvious effect on the change in fungal community structure and composition; however, the changes in AP, OC, AN, TN, and TP had a relatively low impact (Fig. 5b).

At the bacterial phylum level, all bacterial phyla with an average relative abundance greater than 0.5 % exhibited different degrees of negative correlations with changes in TN, CN and OC, particularly the phyla OD1, Verrucomicrobia, Gemmatimonadetes, Actinobacteria, and Acidobacteria. The phyla Nitrospirae, WS3, Firmicutes, Chloroflexi, Proteobacteria, Actinobacteria, and Acidobacteria possessed a strongly positive association with SS, AN, NN and AP, while Cyanobacteria, Planctomycetes, and Bacteroidetes were strongly negatively associated. Additionally, Nitrospirae, WS3, Firmicutes, Chloroflexi, Proteobacteria, Acidobacteria, Actinobacteria, and Gemmatimonadetes revealed a strongly negative association with pH and SM, while Cyanobacteria, Planctomycetes, and Bacteroidetes had a strongly positive association. Nitrospirae and WS3 were positively associated with TP, whereas Verrucomicrobia, Bacteroidetes, and Planctomycetes were strongly negatively correlated with TP (Fig. 5a). At the fungal phylum level, Ichtyosporea was negatively correlated with TP, pH, and CN, while there was a distinctly positive correlation with SS, TN, AN, NN and AP. Basidiomycota, Blastocladiomycota, and Chytridiomycota showed different degrees of negative correlations with TN, AN, OC, TP, AP, and SM, while Blastocladiomycota and Chytridiomycota were positively correlated with pH. Basidiomycota and Chytridiomycota were positively correlated with SS and NN, whereas Ascomycota was positively correlated with TN, AN, OC, TP, AP, and SM. Additionally, Zygomycota was negatively correlated

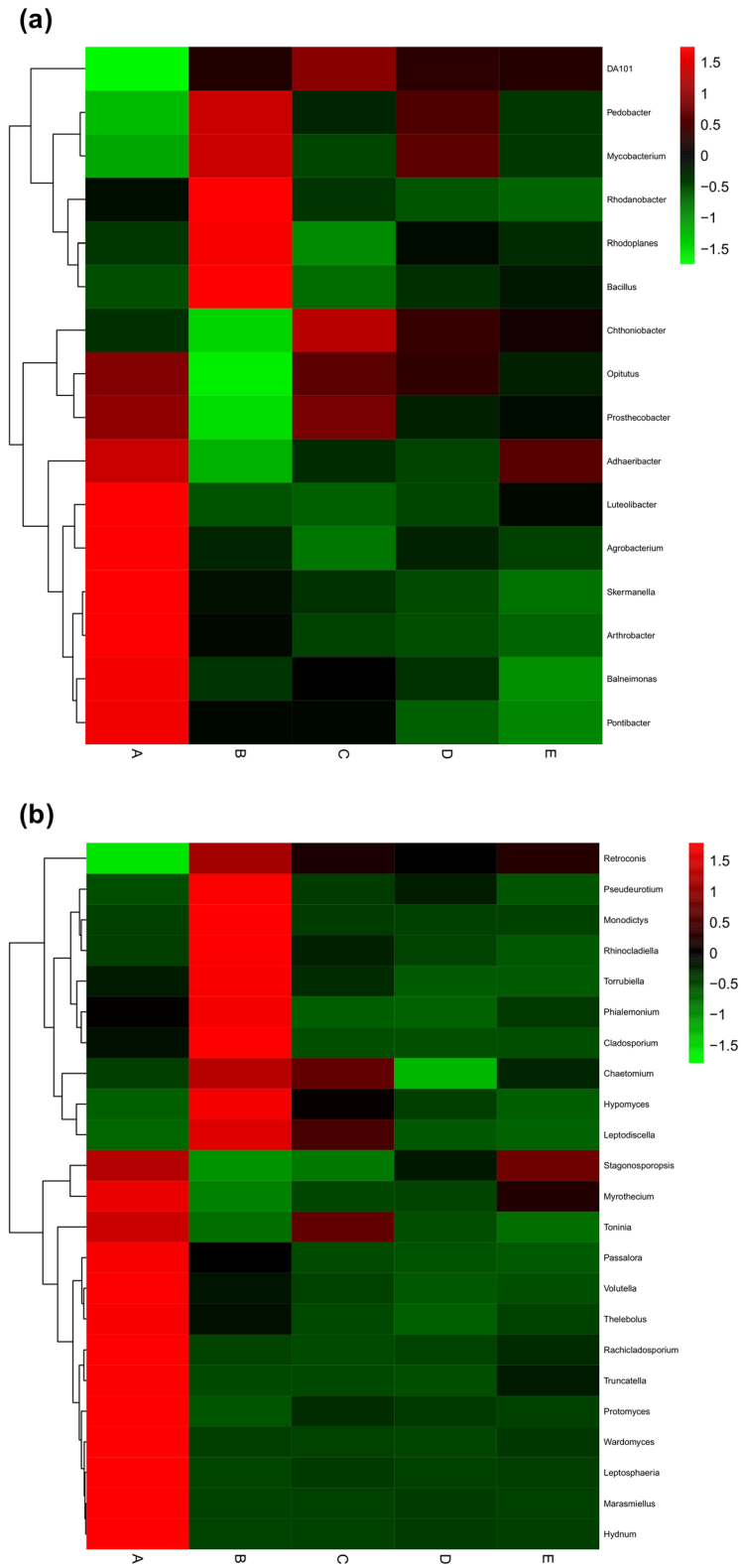


Fig. 4 Heatmap of weighted Bray-Curtis with hierarchal clustering of some significantly different bacteria (**a**) and fungi (**b**) from the soil of farmland that never planted American ginseng (Group A) and recently harvested American ginseng (Group B) at the genus level. The relative abundance for each genus is depicted by color intensity in each field. Higher values are represented by red whereas lower values are represented by green. A, B, C, D, E represented the five groups

with pH and TP, while there was a positive correlation with TN, AN, and AP (Fig. 5b).

According to the results of the RDA, the bacterial genera showed significant differences between groups A and B. *Pedobacter*, *Rhodanobacter*, *Rhodoplanes*, and *Bacillus* were strongly positively associated with SE, TN, AN, NN, and AP, whereas *Opitutus*, *Prosthecobacter*, *Adhaeribacter*, and *Balneimonas* demonstrated a strongly negative association. *DA101*, *Pedobacter*, *Mycobacterium*, *Rhodanobacter*, *Rhodoplanes*, and *Bacillus* were strongly negatively correlated with pH and CN, whereas *Adhaeribacter*, *Luteolibacter*, *Skermanella*, and *Balneimonas* were strongly positively correlated (Fig. 5c). Simultaneously, at the fungal genus level, all of the most significantly increased fungi in group B, in contrast to group A, were negatively correlated with pH, CN, TP, and SM to varying degrees. Furthermore, all significantly decreased fungi were positively associated with these variables, except for *Monodictys*, which was slightly positively associated with TP and had no relationship with CN. Conversely, all significantly decreased fungi in group B compared with group A were negatively correlated with SS, AN, and NN, whereas all significantly increased fungi were positively correlated. Furthermore, except for *Retroconis*, *Chaetomium*, *Leptodiscella*, and *Toninia*, which were negatively correlated with TN, all of the significantly changed fungi were positively associated with TN. In addition, *Retroconis*, *Rhinocladiella*, *Phialemonium*, *Chaetomium*, *Leptodiscella*, and *Toninia* were negatively correlated, while *Torrubiella* and *Hypomyces* had no relevance to SOC, and other significantly changed fungi were positively associated with SOC (Fig. 5d).

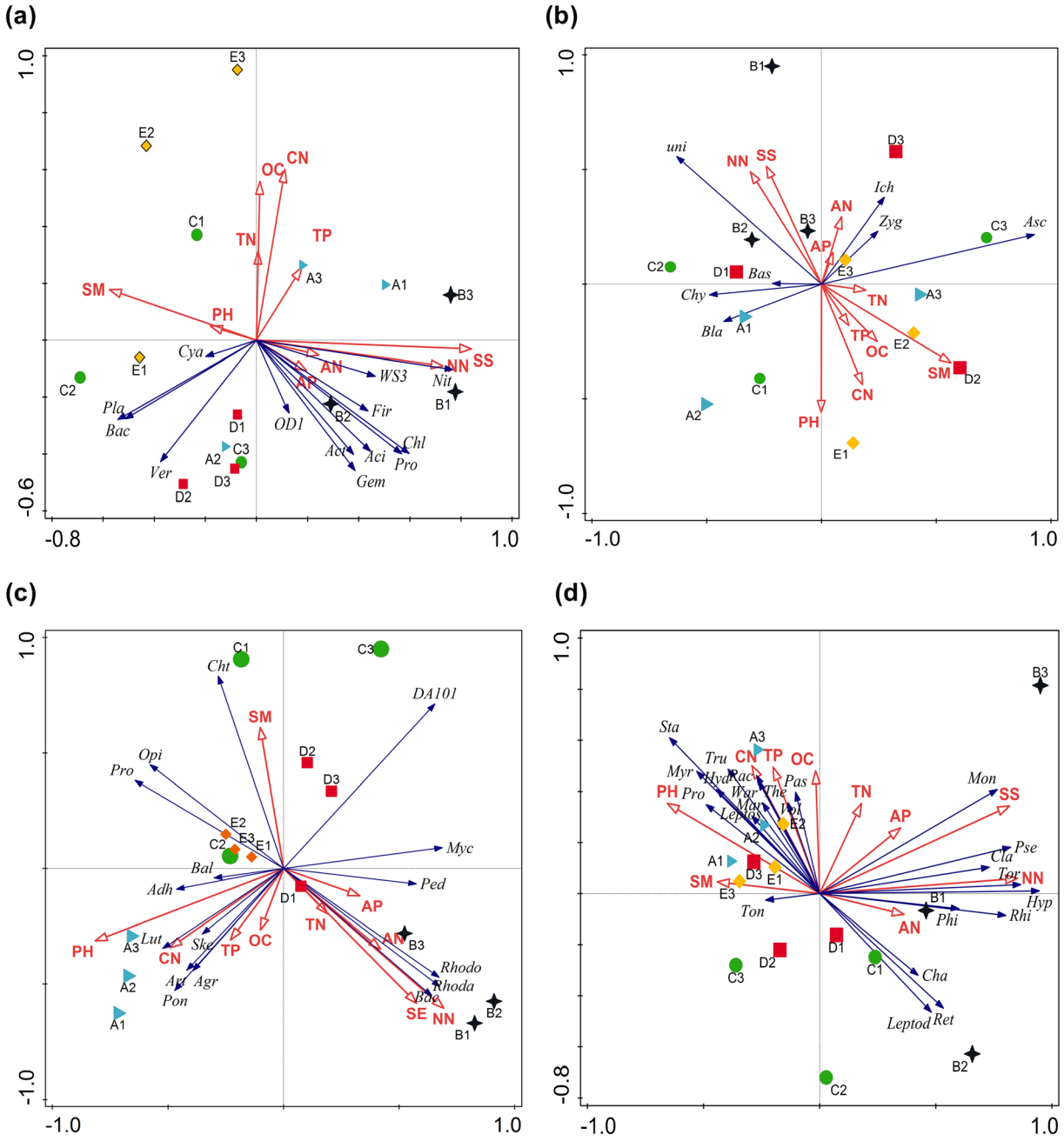
Discussion

Soil pH, salt content, and nitrogen content are important physicochemical factors affecting continuous cropping obstacle of American ginseng

Analysis of the changes in soil physicochemical properties showed that SE, SS, NN, and pH played extremely important roles in the continuous cropping problem of American ginseng. The obstacles to continuous cropping are likely attributable to the decrease in soil pH and the increase in SE, SS, and NN levels associated with the cultivation process.

Our results indicate that soil pH of group B was remarkably lower ($p < 0.01$) than that of group A and gradually recovered with the increasing length of replanting maize time (Fig. 1b). This is consistent with previous researches (Zhang et al. 2020; You et al. 2015) and suggest that the decrease in soil pH is a vital factor in continuous cropping obstacles of American ginseng. Furthermore, because previous studies have reported that the root weights of American ginseng in soils with continuous cropping 2-, 3- and 4-years decreased by 24 %, 17 % and 32 % respectively (Zhao 2009). The seedling survival rate of American ginseng after cropping rotation maize and wheat for 5 years increased from 30 % to about 85 % (Zhang 2013). We speculated that the decrease in soil pH may be an important reason for the decline in American ginseng production. Additionally, we believe that the soil pH of fields where replanting maize after harvesting American ginseng may gradually increase and approach the pH of group A as time length increases, because the soil pH had begun to rise significantly when the farmland was replanted maize for 4-years.

Soil pH has the capacity to impact all chemical, physical, and biological soil properties (Brady et al. 2008; Kemmitt et al. 2006). The present study revealed that the soil pH was similarly correlated with SOC, TN and CN (Pietri and Brookes 2008) and related to changes in the quantity of released NH_4^+ and NO_3^- in soil (Feizi et al. 2017; Nicol et al. 2008). Our work demonstrated that the soil pH was negatively associated with TN, AN and NN (Table S3). These results were consistent with a previous study (Curtin et al. 1998). Additionally, our experiments also demonstrated that TN ($p < 0.01$), AN ($p < 0.05$) and NN ($p < 0.01$) were significantly increased for recently planted American ginseng and were generally restored after replanting maize (Fig. 1f, g, h). These results indicated that changes in soil pH caused by planting American ginseng may cause changes in soil nitrogen content since primary transformations of N in soil-plant systems are affected by NH_4^+-N and NO_3^--N concentrations (De Klein and Van Logtestijn 1994). Organic N mineralization contributes to changes in soil pH values by initially consuming H^+ during the ammonification process, followed by the release of H^+ during nitrification (Xu et al. 2006). Our research appeared to demonstrate this, as soil pH was negatively associated with NN. These results suggested that changes in TN, NN, and AN are material factors affecting the continuous cropping obstacles related to



American ginseng. In association with the changes in TN, CN decreased significantly following the cultivation of American ginseng, which was generally restored to the control level after replanting maize (Fig. 1k). These results indicate that more attention should be paid to the application of N fertilizer when planting American ginseng. CN was also shown to be an important factor in regulating the composition of soil microbial communities and was related to soil pH (Bengtsson et al. 2003; Lovett et al. 2002). This explains the positive correlation between CN and soil pH ($r = 0.632$, $p < 0.05$) (Table S3).

Furthermore, in our study, the SE and SS of group B were remarkably higher than group A ($p < 0.01$) and

Fig. 5 Redundancy analysis of dominant bacterial phyla (**a**), dominant fungal phyla (**b**) (average relative abundance > 0.5 %), significantly different bacterial genera (**c**) and significantly different fungal genera (**d**) across all of the farmland soil samples. Phyla and genera are indicated by blue vectors and physicochemical variables are represented by red vectors. The positions and lengths of the arrows indicate the directions and strengths, respectively, of the effects of variables on microbial communities. Samples were analyzed in triplicate plots. Blue right triangles represent never planted American ginseng farmland (Group A). Black stars represent recently harvested American ginseng farmland (Group B). Whereas orange diamonds, green circles and red squares represent replanted maize for 2 years (Group C) ,4 years (Group D) and 6 years (Group E) after planting American ginseng, respectively. Abbreviations in **a**, Aci, Acidobacteria, Act, Actinobacteria, Bac, Bacteroidetes, Chl, Chloroflexi, Cya, Cyanobacteria, Fir, Firmicutes, Gem, Gemmatimonadetes, Nit, Nitrospirae, Pla, Planctomycetes, Pro, Proteobacteria, Ver, Verrucomicrobia. Abbreviations in **b**, Ich, Ichtyosporea, Asc, Ascomycota, Bas, Basidiomycota, Bla, Blastocladiomycota, Chy, Chytridiomycota, Zyg, Zygomycota, uni, unidentified. Abbreviations in **c**, Ped, *Pedobacter*, Myc, *Mycobacterium*, Rhoda, *Rhodanobacter*, Rhodo, *Rhodoplanes*, Bac, *Bacillus*, Cht, *Chthoniobacter*, Opi, *Opitutus*, Pro, *Prosthecobacter*, Adh, *Adhaeribacter*, Lut, *Luteolibacter*, Agr, *Agrobacterium*, Ske, *Skermanella*, Art, *Arthrobacter*, Bal, *Balneimonas*, Pon, *Pontibacter*. Abbreviations in **d**, Ret, *Retroconis*, Pse, *Pseudeurotium*, Mon, *Monodictys*, Rhi, Rhinocladiella, Tor, Torrubiella, Phi, *Phialemonium*, Cla, *Cladosporium*, Cha, *Chaetomium*, Hyp, *Hypomyces*, Lep, *Leptodiscella*, Sta, *Stagonosporopsis*, Myr, *Myrothecium*, Ton, *Toninia*, Pas, *Passalora*, Vol, *Volutella*, The, *Thelebolus*, Rac, *Rachicladosporium*, Tru, *Truncatella*, Pro, *Protomyces*, War, *Wardomyces*, Lept, *Leptosphaeria*, Mar, *Marasmiellus*, Hyd, *Hydnum*

were restored to the level of group A after replanting maize for 2 years ($p < 0.01$) (Fig. 1c, d). This result suggests that the increase in soil salt content associated with the continuous cropping obstacle of American ginseng and may be reversible. There have been numerous previous studies to illustrate that the soil salt content is important for plant growth (Omer 2004; Wang et al. 2008). The present study showed that crops may develop best when the surface soil salt content is < 0.5 g/kg. Crop growth is restricted when the soil salt content is between 0.5 and 4 g/kg, and little crop growth occurs when the soil salt content is greater than 4.0 g/kg (Jin et al. 2012). It was confirmed in our research that the cultivation of American ginseng contributes to a significant increase in the soil salt content, recording levels that ranged from less than 0.4 g/kg in soils of group A to close to 1.0 g/kg in soils of group B. The soil salt content decreased significantly to the level before planting American ginseng, and it was maintained at this level with a longer time to replant maize. This result indicates that the development of effective methods to reduce the soil salt content following the cultivation of American ginseng may assist in alleviating the continuous cropping problem of American ginseng.

Table 1 Adonis analysis of bacterial and fungal based on UniFrac distances

	Unweighted		Weighted	
	F.Model	Pr (>F)	F.Model	Pr (>F)
Bacteria	2.5421	0.001	2.1427	0.02
Fungi	1.4022	0.01	1.3281	0.107

Data are means ± standard deviation (n = 3)

Soil cellulase and alkaline phosphatase are important soil enzymes affecting continuous cropping obstacle of American ginseng

Biochemical parameters include multiple indicators, and soil enzyme activities are employed as the most important soil quality and fertility indicators (Bastida et al. 2008; Stott et al. 2010). In our study, neutral phosphatase activity (Fig. 2a) was significantly increased ($p < 0.01$) in the soil under the continuous cropping of American ginseng compared with the other groups. However, the alkaline phosphatase (Fig. 2b) and cellulase (Fig. 2d) activities were significantly decreased ($p < 0.01$).

The CN and the types of C and N sources, as well as their individual concentrations, are important parameters in cellulase production. Several studies have shown that the cellulase enzymes might influence microbial activity (Torres et al. 2016), the reduction in cellulase activity associated with continuous cropping obstacle is related to *Myrothecium*, *Volutella*, and *Thelebolus*, which are known to participate in the generation of cellulase activity (Kidder III and Goddard 1965; Singh et al. 2016). The results of our experiments revealed that relative abundance of *Myrothecium*, *Volutella*, and *Thelebolus* were significantly decreased in group B. Levels of *Myrothecium* and *Thelebolus* were gradually restored, although they did not recover to the level of group A (Fig. S7 a, d, g). This explained the positive correlation between cellulase activity and the relative abundance of *Myrothecium* ($r = 0.579$, $p < 0.05$), *Volutella* ($r = 0.324$, $p = 0.240$), and *Thelebolus* ($r = 0.314$, $p = 0.255$) (Table S4). Additionally, changes in the relative abundance of *Chaetomium* were

significantly negatively correlated with cellulase activity (r = -0.653, $p < 0.01$). *Chaetomium* is an important fungus that is contributed to decompose cellulose and resides within various plant residues, cellulose-rich substrates and soils (Hubbard et al. 2011; Lee and Hanlin 1999). Here, the cellulase activity gradually recovered with increasing replanting maize time. This suggests that cellulase activity is a key factor that affects the continuous cropping problem of American ginseng, which was impacted by changes in soil microbial community.

Alkaline phosphatase (AlkP) activity was significantly decreased in group B compared with group A; AlkP declined in group C and then gradually recovered (Fig. 2b). Previous studies have found that AlkP increases as the soil pH increases (Dick et al. 2000). These reports articulated that alkaline phosphatase was positively correlated with soil pH ($r = 0.910$, $p < 0.01$) (Table S5). Combined with the influence on the soil microbial community (Acostamartinez et al. 2003), we believe that alkaline phosphatase is another crucial extracellular enzyme that affects the continuous cropping of American ginseng and is closely related to changes in microbial community structure and composition.

Changes in soil microbial community are closely related to changes in soil physicochemical and enzyme activities

For this study, we characterized the changes in microbial community structure and composition of farmlands soils from which planted with American ginseng and replanted maize after American ginseng. Our research suggests that the bacterial diversity of soil tends to be lower following the planting of American ginseng, while the fungal diversity and bacterial and fungal richness both tend to be higher. Furthermore, Pearson's correlation analysis between the alpha diversity index and soil pH showed that soil pH was positively correlated with bacterial diversity ($r = 0.535$, $p < 0.05$), but it was negatively correlated with fungal diversity ($r = -0.080$, $p = 0.777$) (Table S6). This is consistent with the findings of previous studies (Dong et al. 2017; Rousk et al. 2010; Zheng et al. 2019).

Specifically, at the bacterial phylum level, bacterial community composition was dominated by Bacteroidetes, Proteobacteria, Planctomycetes, Acidobacteria, Actinobacteria, etc. This was consistent with previous studies (Wolińska et al. 2017; Yu et al. 2018; Zheng et al. 2019). Acidobacteria is believed to have an extensive range of metabolic and genetic functions (Kielak et al. 2016). Soil pH is one of the key factors that influences the composition and structure of Acidobacteria communities (Sait et al. 2006; Zhang et al. 2014) and Acidobacteria exhibited a robust, inverse response to soil pH (Zheng et al. 2019). The relative abundance of Acidobacteria has been shown to increase with lower soil pH (Dimitriu and Grayston 2010; Rousk et al. 2010). According to the RDA, our study also demonstrated that Acidobacteria was strongly negatively correlated with soil pH (Fig. 5a). In comparison, Nitrospirae (0.8 %) and WS3 (0.9 %) significantly increased in soils planted with American ginseng ($p < 0.01$) and gradually recovered to pre-cultivation levels after replanting maize (Fig. 6). This result indicates that increases in Nitrospirae and WS3 are potentially important factors in the continuous cropping problem of American ginseng. Furthermore, our study reveals that the relative abundance of Nitrospirae is positively correlated with AN ($r = 0.515$, $p < 0.05$) and NN

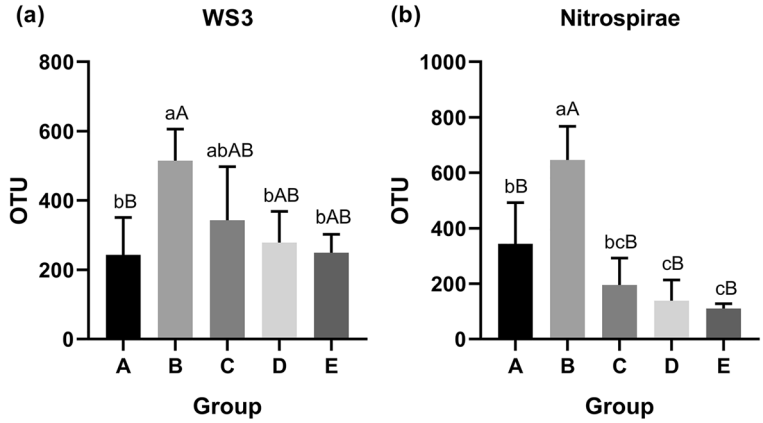


Fig. 6 Significantly different bacterial phyla of recently harvested American ginseng farmland (Group B) compared with never planted American ginseng farmland (Group A). Data are means ± standard deviation (n = 3). Different letters (a, b, c) indicates a significant difference from each other at p ≤ 0.05, and different letters (A, B, C) indicates a remarkable difference from each other at p ≤ 0.01

($r = 0.840$, $p < 0.01$) (Table S7, Fig. 5a). These results are in alignment with a previous study, in which the higher abundance of Nitrospirae reinforces the idea of a community adapted to improved N mining in these environments (Carbonetto et al. 2014).

At the bacterial genus level, the relative abundance of *Bacillus* was significantly increased following the planting of American ginseng. Most *Bacillus* members can inhibit the propagation of pathogenic microbes in soils; additionally, they can invade the roots of plants, reduce soil-borne diseases in plants, regulate and balance soil pH, adjust the ecological environments of plant roots, and form dominant colonies to remediate the damage inflicted on soils by chemical fertilizers, pesticides, and other harmful factors (Lin et al. 2014; Shafi et al. 2017). Consequently, increased *Bacillus* may be a response to changes in the physicochemical properties and microbial community structures and compositions of soils, particularly in terms of decreasing pH, increasing SS, and harmful bacteria and fungi. *Opitutus* is believed to contribute to the N cycle by reducing nitrate to nitrite, which other bacteria convert further (Chin et al. 2001). Our results also reveal that changes in the relative abundance of *Opitutus* were significantly negatively correlated with TN ($r = -0.560$, $p < 0.05$), AN ($r = -0.580$, $p < 0.05$), and NN ($r = -0.718$, $p < 0.01$). Our results show that some Proteobacteria, such as *Agrobacterium*, *Balneimonas*, and *Skermanella*, were significantly decreased. In addition, there were other beneficial bacterial genera that also significantly decreased following the planting of American ginseng. For example, *Chthoniobacter* was found to improve the health of soils (Sangwan et al. 2004).

At the fungal phylum level, fungal communities were dominated by Ascomycota, Basidiomycota, and Zygomycota, which is consistent with the findings of previous studies (Egidi et al. 2019; Huang et al. 2015; Tan et al. 2017). We found that almost all the significantly altered fungal genera in group B (compared with group A) were members of Ascomycota and Basidiomycota. Previous studies revealed that the members Ascomycota and Basidiomycota were primary soil fungal decomposers (Bastian et al. 2009). The decomposition of crop residues by soil microbial communities is a key step toward the release of inorganic nutrients from plant residues (Ma et al. 2013). We found that almost all significantly increased fungal genera in group B, in contrast to group A, were significantly positively correlated with SE, SS, AN, and NN (Table S8). Furthermore, the soil SE and SS were significantly positively correlated with *Torrubiella* ($r = 0.889$, $p < 0.001$), *Cladosporium* ($r = 0.849$, $p < 0.001$), *Rhinocladiella* ($r = 0.874$, $p < 0.001$), and *Phialemonium* ($r = 0.904$, $p < 0.001$), whereas the soil NN was significantly positively correlated with *Torrubiella* ($r = 0.900$, $p < 0.001$), *Rhinocladiella* ($r = 0.907$, $p < 0.001$), and *Cladosporium* ($r = 0.896$, $p < 0.001$).

A previous study reported that soil pH positively affected plant diversity in regions with predominately neutral soils but negatively affected plant diversity in regions where acidic soils dominated (Partel 2002). This confirmed that the most significantly changed fungi were strongly related to soil pH. As such, the soil pH was significantly positively correlated with *Myrothecium* ($r = 0.748$, $p < 0.01$), *Truncatella* ($r = 0.820$, $p < 0.001$), and *Protomyces* ($r = 0.766$, $p < 0.001$), while it was significantly negatively correlated with *Chaetomium* ($r = -0.525$, $p < 0.05$), *Cladosporium* ($r = -0.583$, $p < 0.05$), and *Leptodiscella* ($r = -0.567$, $p < 0.05$). The presence of *Myrothecium* is extensive in plants and soil, with most species having a strong capacity to decompose cellulose. Some species can produce antibiotics, which is related to a decrease in cellulase activity (Brian et al. 1948; Brian and Mcgowan 1946). Our results showed that changes in the relative abundance of *Myrothecium* were significantly positively correlated with cellulase activity ($r = 0.579$, $p < 0.05$). As an important cellulolytic fungus, *Chaetomium* plays a critical role in the carbon cycle and soil improvement in natural ecosystems, and many species have beneficial biocontrol potential in agricultural production. Changes in the relative abundance of *Chaetomium* were significantly negatively correlated with soil pH ($r = -0.525$, $p < 0.05$) and positively correlated with SS ($r = 0.573$, $p < 0.05$) in response to decreased soil pH and increased SS. Additionally, as a potential biocontrol fungus, changes in the relative abundance of *Hydnum* were significantly decreased in group B compared with group A, significantly negatively correlated with soil pH ($r = -0.634$, $p < 0.05$), and positively correlated with SS ($r = 0.728$, $p < 0.01$). A study has shown that members of *Hydnum* might be associated with the symptoms of root rot for a broad array of woody and herbaceous host plants (Bastos et al. 1981).

In summary, we believe that the change in bacterial and fungal community structure is an important factor that leads to the failure of continuous cropping for

American ginseng; balancing soil pH may be an essential prerequisite to improve the diversity and abundance of fungal communities to resolve the continuous cropping problem of American ginseng. A number of effective measures could be performed in actual cultivation applications, such as the addition of organic amendments, which have proven to be a viable strategy for the regulation of soil pH, nitrogen availability, and microbial community composition (Bowles et al. 2014; Feizi et al. 2017). Furthermore, fertilization with wood ash and lime has the capacity to dramatically increase and improve the soil pH over time to levels that are suitable for plant growth (Demeyer et al. 2001; Fritze et al. 1994).

Conclusions

In this study, we collected soils from maize fields where American ginseng had never been planted, from fields where American ginseng had just been harvested, and from fields where maize had been planted for 2, 4 and 6 years after harvesting American ginseng. We analyzed the physical and chemical properties, enzymatic activities, and microbial community structure and composition. We found that a significant decrease in soil pH and a substantial increase in soil salt content were critical factors that led to the continuous cropping problem of American ginseng. According to our results, the modification of these physical and chemical properties caused significant changes in microbial community structures, as well as changes in soil bacterial and fungal diversity and composition following the cultivation of American ginseng. Furthermore, the relative abundance of some bacteria, such as Nitrospirae, *Opitutus*, *Agrobacterium*, *Balneimonas*, and *Skermanella*, were altered, as were the relative abundance of associative fungal communities, such as *Myrothecium*, *Chaetomium*, *Volutella*, and *Thelebolus*. Pearson's correlation analysis shown that these changes were influenced by the alteration of soil physical and chemical properties. Soil cellulase and alkaline phosphatase activities were also changed following the cultivation of American ginseng.

In summary, changes in soil pH, soil salt content and nitrogen content lead to changes in microbial community structure and composition, as well as cellulase and alkaline phosphatase activities, these changes may cause the continuous cropping obstacles of American ginseng and may be improved by replanting maize.

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Author contributions All authors contributed to the study conception and design. Conceptualization: Shuai Liu, Jun-feng Niu and Shi-qiang Wang; Methodology: Shuai Liu and Shu-ke Zhang; Writing-Original Draft: Shuai Liu; Formal analysis: Shuai Liu, Zhan-yu Wang and Kai-Kai Dang; Resources: Shuai Liu and Shi-qiang Wang; Data Curation: Zhan-yu Wang, Kai-Kai Dang and Shu-ke Zhang; Investigation: Zhan-yu Wang and Kai-Kai Dang; Funding acquisition: Jun-feng Niu, Shi-qiang Wang and Zhe-zhi Wang; Writing- Reviewing and Editing: Jun-feng Niu and Zhe-zhi Wang. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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