Dynamic succession of soil microbial community during continuous cropping of *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.)

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Background. Continuous cropping disturbs the balance between the microbes beneficial to a plant and the pathogenic microorganisms in the rhizosphere soil, which has both a direct and indirect adverse effect on soil and plant health. It is highly significant to understand the mechanism of the obstacle found in continuous cropping and to search for a reasonable rotation model to solve the problem of continuous cropping. *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) (*A. mongholicus*) is a critical traditional Chinese herb, which is negatively affected by continuous cropping. Previous studies on the root rot pathogens of *A. mongholicus* have been conducted, while reports on the effects of *A. mongholicus* on the health of soil affected by continuous cropping are lacking.
Methods. In this study, we observed the microbial community structure and the diversity of the rhizosphere soil under continuous cropping for 1, 3, and 6 years using the pyrosequencing approach, and compared this to bulk soil, using *A. Mongholicus* as the experimental material. The 16S rDNA and ITS amplicon sequencing techniques were used to detect the composition and diversity of bacteria and fungi in the rhizosphere soil and the bulk soil of *A. Mongholicus*. The diversity of the bacterial community and the structures of the rhizosphere and bulk soils were compared. The dynamics of the soil enzyme activity were also analyzed. **Results.** The results of this study illustrated that the continuous cropping of *A. mongholicus* caused a decline in the root dry weight, the ratio of root-top, and also influenced the growth of the root system of *A. mongholicus*. Continuous cropping and the sampling time shifts the diversity and structure of the microbial community in the rhizosphere soil of *A. mongholicus*, showing that the diversity of the microbial community in the *A. mongholicus* rhizosphere soil was decreased with an increase in the replanting years, while the structure of the microbial community deteriorated. The relative abundance of pathogenic fungi, *Fusarium*, *Erysiphe*, *Rhizobiales,* and *Burkholderiales* as well as bacteria related to nodulation were enriched in the *A. mongholicus* rhizosphere soil at different sampling stages. The beneficial bacteria decreased with the increasing years

of continuous cropping during growth, which resulted in the microecological imbalance in the *A. mongholicus* rhizosphere, caused serious replanting diseases of continuous cropping. A decline in soil urease and invertase activities was observed after 6 years of continuous cropping. Our experimental results suggest that continuous cropping has a significant impact on soil bacterial and fungal community development, and that an increase in replanting years resulted in more negative impact on rhizosphere soil health and *A. mongholicus* growth.

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Abstract

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Results. The results of this study illustrated that the continuous cropping of *A. mongholicus* caused a decline in the root dry weight, the ratio of root-top, and also influenced the growth of the root system of *A. mongholicus*. Continuous cropping and the sampling time shifts the diversity and structure of the microbial community in the rhizosphere soil of *A. mongholicus*, showing that the diversity of the microbial community in the *A. mongholicus* rhizosphere soil was decreased with an increase in the replanting years, while the structure of the microbial community deteriorated. The relative abundance of pathogenic fungi, *Fusarium*, *Erysiphe*, *Rhizobiales,* and *Burkholderiales* as well as bacteria related to

nodulation were enriched in the *A. mongholicus* rhizosphere soil at different sampling stages. The beneficial bacteria decreased with the increasing years of continuous cropping during growth, which resulted in the microecological imbalance in the *A. mongholicus* rhizosphere, caused serious replanting diseases of continuous cropping. A decline in soil urease and invertase activities was observed after 6 years of continuous cropping. Our experimental results suggest that continuous cropping has a significant impact on soil bacterial and fungal community development, and that an increase in replanting years resulted in more negative impact on rhizosphere soil health and *A. mongholicus* growth.

Introduction

The rhizosphere is a micro-environment of plant root systems and soil interface. It is where the soil-root-microorganisms interact closely and influence each other (*Micallef et al., 2009*). It is home to an overwhelming number of microorganisms and invertebrates and is considered one of the most dynamic interfaces on earth (*Philippot et al., 2013*). The community structures and changes in the microorganisms of the rhizosphere reflect the productivity and stability of the soil (*Waldrop et al., 2000*; *Nannipieri et al., 2003*). The rhizosphere's microorganisms provide organic nutrients for plants by converting organic compounds into inorganic substances (*Mendes et al., 2011*). Furthermore, the secretion of vitamins, growth hormones, and other substances regulate the bacterial activity of the plant pathogens, thereby directly affecting the growth and health of the plants (*Kardol et al., 2007*). Plants can secrete 40% of their photosynthate into the rhizosphere soil (*Costa et al., 2006*), providing maximal carbon sources for heterotrophic soil organisms and serving as a vital factor in determining the structure of the rhizosphere microbial communities (*Bakker et al., 2014*). The root exudates and residue decomposition of plants directly affects the population structure and numbers of microorganisms in the rhizosphere (*Berendsen et al., 2012*). Subtle changes in the plant genotypes can also lead to distinct differences in rhizosphere microbiota (*Lundberg et al., 2012*; *Bulgarelli et al., 2012*). A specific microbial community enriches each plant's rhizosphere soil and many plant pathogenic microorganisms, bacteria, and fungi exert synergistic effects on plants, displaying a high specificity for the host (*Yao et al., 2000*). Like the rhizosphere soil microorganisms, the soil's enzyme activity is a biological indicator of soil health. Soil enzymes are mainly derived from microorganisms, plant roots, and soil animals. They can participate in nutrient cycling, organic matter transformation, and humus and colloid formation (*Zhang et al., 2013*). The activity of the soil enzymes reflects the predispositions and intensities of various biochemical processes in the soil (*Guan et al., 1980*).

Several biotic and abiotic factors affect the soil rhizosphere microorganisms and enzyme activities including the plant species, the physical and chemical properties of the soil (*Berg et al., 2009*; *Santos-Gonzalez et al., 2011*), the stage of plant development (*Yuan et al., 2015*), and crop cultivation (*Lupwayia et al., 2007*; *Lienhard et al., 2013*). Reportedly, the continuous cropping of a plant can change the physical and chemical characteristics of the soil, leading to changes in the diverse microbial community in the rhizosphere (*Yao et al., 2006*; *Yoneyama et al., 2010*). Tillage also has a negative effect on soil productivity and plant growth, resulting in soil-borne diseases, which pose a severe threat to agricultural and economic development. Franke-Whittle (*Franke-Whittle et al., 2015*) found that some known pathogens such as *Acremonium,*

Cylindrocarpon, and *Fusarium*, as well as some newly discovered pathogenic bacteria, namely *Chitinophaga, Hyphomicrobium, Nitrosospira*, might be related to the occurrence of apple replant disease, based on the sequencing analysis of the 16S rRNA V1–V3 and ITS1 regions. Moreover, beneficial bacteria (*Gp16* and *Solirubrobacter*) and fungi (*Penicillium* and *Paecilomyces*) were reduced in replanted soil, indicating that during continuous cropping, there is an increase of pathogenic bacteria and a reduction of beneficial bacteria. Chen (*Chen et al., 2014*) also found that *Alteromonadales, Burkholderiales, Flavobacteriales, Pseudomonadales, Rhizobiales,* and *Rhodospirillales*, bacteria which are beneficial for the plants were reduced, resulting in decreased peanut production. Under continuous cropping, some microorganisms, such as *Acidobacteriales, Chromatiales,* and *Gemmatimonadales* increase with the growth period of the peanut (*Chen et al., 2012*). Another study on continuous cropping of cotton showed that alterations in root exudates affected the microbial composition around plants through the interaction between plant and microorganism. In contrast, *F. oxysporum f. sp. Vasinfectum,* and *V. dahlia* were enriched and there was a lessening of pathogenic antagonistic *Xanthomonadaceae, Comamonadaceae, Oxalobacteraceae,* and *Opitutaceae. This* This would explain the negative effects of the continuous cropping of cotton (*Li et al., 2015*). Therefore, under continuous cropping, some pathogenic and beneficial microorganisms significantly affect the soil microbial community succession process in the rhizosphere.

Astragalus membranaceus Bge. var. *mongholicus* (Bge.) Hsiao is an economically important, medicinal plant commonly used in traditional Chinese medicine (TCM). It contains amino acids, trace elements, polysaccharides, and other components (*Wu et al., 2005*; *Liu et al., 2012*) with a high nutritional value and healthy function, according to the 2015 edition of Chinese Pharmacopoeia. It is also reported to improve resistance against disease in humans. *A. mongholicus* is primarily distributed in the north and northwest of China (*Jia et al., 2016*) with some wild resources found in Mongolia and Korea. Owing to market demand and limitations of cultivated land area, *A. mongholicus* is often replanted for several years in the same area, causing reduced yield, quality, and an increased incidence of root rot (*Chen et al., 2014*). The present study aims to determine the phylogenetic affiliations of the most common and predominant populations of soil bacteria and fungi at different sampling stages under a continuous cropping regime. Moreover, it also identifies the diversity and succession patterns of soil microorganisms and enzyme activity as the years of continuous cropping increase.

Materials & Methods

Experimental Design

This study was conducted at the Inner Mongolia University (40°48’ N, 111°40’ E, elevation 1040 m), Hohhot City, Inner Mongolia Province, Northwest China. *Astragalus membranaceus* Bge. var. *mongholicus* (Bge.) Hsiao seedlings were bought from Jinlvyuan Green Engineering Co., Ltd, Xilin Gol League. To reduce the interference from external factors on the soil microbial community, pot culture experiments were conducted. Soil for the pot culture experiments that corresponded with the different time periods of continuous cropping were collected from the farm of Wuchuan City, Inner Mongolia. The farm was managed by the regular method and soil was kept outside under natural conditions. Naked oats were planted in June and collected in September each year. *A. mongholicus* were planted in May 2010 and 2013 and entered the winter dormancy period after September every year, awakening from dormancy in May of the following

year. Ammonium nitrate-based fertilization, weed control, and pest control were performed according to local recommendations. The farm was weeded artificially three times in July and August of each year. The soil pH was maintained at 8.39 and the soil profile was 28.5% clay, 41.0% loam, and 30.5% sand with 19.30 $g \cdot kg^{-1}$ organic matter, 1.08 $g \cdot kg^{-1}$ total nitrogen, 0.77 $g \cdot kg^{-1}$ total phosphorus, 110 $mg \cdot kg^{-1}$ hydrolyzed nitrogen, 35.8 $mg \cdot kg^{-1}$ of the available phosphorus, and 118 $mg \cdot kg^{-1}$ of the available potassium, and an electrical conductivity (EC) of 0.112 $ms \cdot cm^{-1}$. The physical and chemical properties of the soil samples were analyzed at the Ecological Laboratory of Inner Mongolia University.

The pot culture experiments included four treatments: CC1, transplants of *A. mongholicus* seedlings into soil that had oats growing for one year previously (the soil had grown *A. Mongholicus* for one year); CC3, transplants of *A. mongholicus* seedlings into soil that was continuously cropped for two years (the soil had grown in *A. mongholicus* for three years); CC6, transplants of *A. mongholicus* seedlings into soil that was continuously cropped for five years (the soil had grown in *A. mongholicus* for six years); CK, no *A. mongholicus* transplants were placed into uncultivated field soil, which was obtained adjacent to the other soil samples and used as a control (the soil has not grown *A. mongholicus*). Simultaneously, a five-point sampling method was used to collect the different years of continuous cropping soil. The soil (100 kg fresh weight) was mixed and sieved to remove stones, roots, and other debris. On 10 May 2015, uniformly normal, healthy *A. mongholicus* seedlings that had been rinsed three times with sterile water, were transplanted into clean, sterile plastic pots (17 cm diameter × 25 cm height) filled with 2.5 kg soil. During the experimental period, the average temperature was maintained at 12–18 °C at night and 22–30 °C during the day with natural light, and 60–70% relative water in the soil. The plants were irrigated with sterile water, and no fertilizer was applied during the longest four months of the growing seasons and fallow periods.

Sample collection

The samples were collected at three different *A. mongholicus* growth stages in the four treatments: (i) before transplanting (May), (ii) at the vigorous growth period (July), and (iii) at harvest time (September). *A. mongholicus* plants, sampled in September, were carefully uprooted from the soil and shaken slightly to remove the loosely attached soil. The rhizosphere soil tightly attached to the roots was brushed off and collected (*Wu et al., 2015*). The soils from each pot were then pooled in a Ziploc bag and placed into a cooler on ice. These soil samples were then used for molecular analysis after sieving through a 2-mm sieve, and stored at -80 °C until required for DNA extraction.

Soil enzyme assays

The activities of the soil enzymes invertase and urease were determined according to the method described by Guan *et al* (*Guan et al., 1980*). The invertase activity was measured colorimetrically using 3,5 - dinitrosalicylic acid and expressed in glucose $mg \cdot g^{-1}$ soil at 37 ℃ over 24 h. The urease activity was determined colorimetrically using indophenol blue and expressed in NH_3-N $mg \cdot g^{-1}$ soil at 37 ℃ over 24 h. The cellulose activities were estimated by the method of Schinner and Wvon (*Schinner & Wvon, 1990*) using a spectrophotometer (Shimadzu

UV-2450). The cellulase activity was measured using carboxy methyl and expressed in glucose equivalents (GE) mg·g^{-1} soil at 50 ℃ over 24 h.

DNA extraction, PCR amplification, and Illumina sequencing of bacterial and fungal communities

Four replicate samples were randomly selected for one treatment and used for DNA extraction. Total DNA from the rhizosphere soil samples of *A. mongholicus* was extracted using a Soil DNA Kit, according to the manufacturer's instructions (OMEGA, USA). The extracted soil DNA was solubilized in 100 µL TE buffer and subjected to 1% agarose gel electrophoresis to check for purity, followed by NanoDrop quantification. According to the concentration, DNA was diluted to 1 ng·µL^{-1} using sterile water. The 16S rRNA with V4 region genes of bacteria and archaea were amplified from the genomic DNA using 515F (5'-GTGCCGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGG GTWTCTAAAT-3') primers (*Dethlefsen et al., 2008*). The fungal Internal Transcribed Spacer (ITS) sequences of the hypervariable region were amplified using ITS5-1737F (5'-GGAAG TAAAACAAGG-3') and ITS2-2043R (5'-GCTGCGTTCTTCATCGATGC-3') primers (*Buee et al., 2009*). DNA was amplified according to the protocol described previously (*Magoc et al., 2011*). 30 µL PCR reactions contained 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Singapore), 0.2 µM final concentration of each primer, and 10 ng template DNA. After initial denaturation at 95 °C for 1 min, each thermal cycle consisted of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. At the end of 30 cycles, the final extension step was carried out at 72 °C for 5 min. An equivalent volume of 1X loading buffer (contained SYB green) was added to PCR products and the products were detected by 2% agarose gel electrophoresis. The samples with 400–450 bp (bacteria) and 300-500 bp (fungal) amplified products were selected for subsequent experiments. The PCR products were mixed in equivalent ratios and purified using the GeneJET Gel Extraction Kit (Thermo Scientific, USA). Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer's recommendations, and index codes were added. The quality of the library was assessed on a Qubit 2.0 Fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina HiSeq platform, and about 250 bp paired-end reads were generated for subsequent analysis. The amplicon pyrosequencing was performed on the Illumina MiSeq platforms at Novogene Bioinformatics Technology Co., Ltd (Beijing, China).

Data analysis

After removing the adaptor contamination, primer sequences, and low-quality reads, the raw sequences were assembled for each sample according to the unique barcode using QIIME (Quantitative Insights into Microbial Ecology). The paired-end reads from the original DNA fragments were merged using FLASH (*Magoc et al., 2011*) and assigned to each sample according to the unique barcodes. The sequences were analyzed using the QIIME software package and in-house Perl scripts were used to for assessing the α- (within samples) and β-

(among samples) diversities. Sequences with ≥97% similarity were assigned to the same operational taxonomic unit (OTUs) (*Edgar et al., 2011*).

All data was subject to analysis of variance (ANOVA) using the general linear model-univariate (mixed model) procedure by IBM SPSS Statistics 19.0. The treatments were compared for any significant differences using the LSD multiple range tests at the significance level of P=0.05.

Results

Biomass of *A. mongholicus*

The root dry weight of *A. mongholicus* was decreased with the years of continuous cropping; the weights in CC1 were higher with 35.8% and 51.1% than those in CC3 and CC6, respectively (Fig.1). After 3 years of continuous cropping, the growth of *A. mongholicus* root showed a reducing trend, and was better developed in the soil that had not been treated with replanting. The ratios of the root-top in CC1 and CC3 were also higher with 20% and 16%, respectively, than that of CC6.

Statistical analyses of the sequence data in *A. mongholicus* rhizosphere soils

Twelve 16S rRNA gene libraries were constructed over four continuous cropping treatments with three sampling stages for each treatment. For each treatment, four subsamples from each replicate were analyzed. After filtering based on the basal quality control processing, a total of 3,560,456 bacterial effective tags used for further analyses were obtained from pyrosequencing after filtering low-quality, short-length sequences and the chimeras. The number of effective tags per treatment did not differ greatly between the four soil treatments (Table 1) and were grouped into 239,377 OTUs at 97% sequence similarity level, ranging from 4,293 to 6,158 OTUs for the four treatments. Additionally, a total of 2,894,024 effective fungal tags were obtained, and 28,237 OTUs were identified that varied from 279 to 887 in each treatment (Table 1). The average read length of bacterial and fungal sequences was 254 and 253 bp, respectively. In total, 475 bacterial genera and 405 fungal genera were detected in all soils.

As shown in Table 2, the coverage of all samples, irrespective of bacteria or fungi, were >92.9%, indicating that the sequencing depth met the needs of our experiments (Table 2). The count of the bacterial OTUs were attributed to species (Fig. 2). The largest number of bacterial OTUs (6158) was obtained for the CC6 sampling in May, which was higher than CC3, significantly higher than CC1as well as the bulk soil CK sampling in May. The bacterial OTUs were significantly less for the CC1 sample compared to the CC6 sample in September. The OTUs of CC6 sustained a decline from May to September. During *A. mongholicus* growth, the fungal OTUs in CK (656 in September) were increased from May and differed significantly from the CC1, CC3, and CC6 treatments. Finally, the observed bacterial and fungal species in the continuous cropping soil (i.e CC3 and CC6) were lower than CK and CC1 at the time of harvesting.

Bacterial and fungal community composition of *A. mongholicus* rhizosphere soils

The bacterial pyrosequencing showed that a total of 239,377 OTUs were identified in 12 soil samples, and these sequences were affiliated with 57 bacterial phyla, 153 classes, 239 orders, 316 families, and 475 genera. The *Proteobacteria, Actinobacteria,* and *Acidobacteria* phyla were highly abundant with relatively low abundances of *Bacteroidetes* and *Firmicutes* found in all soils, which is consistent with the previous reports that plants appear to favor the colonization of

their rhizosphere by *Proteobacteria*. The bacterial phyla, *Bacteroidetes, Actinomycetes*, and *Firmicutes* were also commonly observed in both niches (*Philippot et al., 2013*; *Bulgarelli et al., 2013*; *Moore et al., 2015*). The OTUs classified from all soil samples were primarily affiliated with 10 bacterial phyla and 10 fungal phyla (Fig. 3). *Proteobacteria* (31.8% of all sequence reads), *Actinobacteria* (26.9%), *Acidobacteria* (12.8%), *Chloroflexi* (5.91%), *Gemmatimonadetes* (4.19%), *Bacteroidetes* (3.46%), *Planctomycetes* (3.34%), *Firmicutes* (3.10%), *Crenarchaeota* (2.37%), and *Cyanobacteria* (1.42%) were the dominant bacterial phyla and had average relative abundances >1%. The relative abundances of *Firmicutes* phyla decreased with the continuous cropping years of *A. mongholicus* in July. The relative abundances of *Acidobacteria, Chloroflexi, Gemmatimonadetes,* and *Planctomycetes* phyla were decreased with the long-term (ie CC3 and CC6) continuous cropping of *A. mongholicus* in September (Fig. 3A). A comparison of the relative abundances of the top 20 classified bacterial orders showed significant variations among the two-continuous cropping *A. mongholicus* soils and the CK soil (Fig. 3C). *Actinomycetales*, *iii1-15*, *Rhizobiales, Sphingomonadales, Burkholderiales, Nitrososphaerales, Solirubrobacterales*, and 19 other bacterial orders were dominant at the three sampling time points and in the soils (average relative abundances >1%). With long-term continuous cropping, the relative abundances of *Rhizobiales* (6.7% in September) were increased. The relative abundances of *Sphingomonadales* (4.5% in September) were increased followed by a decrease, and the maximum relative abundance was reached in the CC3 soil (5.2%). However, the relative abundances of *Solirubrobacterales* (3.3–3.5%) and *Burkholderiales* (3.8–4.8%) were increased continuously from CC1–CC6. In addition, some specific bacterial families were dominant in different sampling periods and the continuous cropping soils.

Among the fungi in all the soil samples, a total of 26,237 OTUs were affiliated with 12 fungal phyla, 30 classes, 90 orders, 191 families, and 405 genera. Several reports investigating the fungal communities of soil using deep amplicon sequencing in peanut, pea, and vanilla crops showed *Ascomycetes* as the predominant fungal phylum, followed by *Basidiomycetes* (*Jumpponen et al., 2010*; *Xu et al., 2012*; *Li et al., 2014*). *Ascomycetes* constituted the largest group of pathogens (*Lu et al., 2003*) and the antimicrobial activities of *Basidiomycetes* strains had frequently been reported (*Srivastava et al., 2011*). Our results show that the dominant fungal phyla (relative abundance >1%) across all samples included *Ascomycota* (91. 2% of all sequence reads), *Basidiomycota* (2.95%), and *Zygomycota* (1.67%) accounting for 95.8% of the fungal sequences. In addition, *Chytridiomycota* (0.80%) and *Glomeromycota* (0.02%) were present in all samples in low relative abundance (relative abundance ranged between 0.01% and 0.10%). The relative abundance of *Chytridiomycota* phyla increased with the continuous cropping years of *A. mongholicus* in May. The relative abundances of *Cercozoa* phyla were decreased with the continuous cropping years of *A. mongholicus* in July. The *Neocallimastigomycota* and *Ciliophora* phyla were only identified in the CK soil (Fig. 3B). On the order level, *Hypocreales, Pleosporalse, Capnodiales, Sordariales, Pezizales, Cantharellales, Ustilaginales, Mortierellales, Microascales, Xylariales, Agaricales* and three other orders were predominant at the three

sampling time points and in all soils (average relative abundance >1%). With long-term continuous cropping, the relative abundance of *Hypocreales* in the CK soil was significantly higher than the replanted soils in May (5.6%) and September (16.9%). The relative abundance of *Pleosporalse* was decreased, while that of *Pezizales* was increased in July (Fig. 3D). Significant differences were observed in the relative abundance of fungal orders, such as *Erysiphales* (0.005–21.0%), *Mortierellales* (0.2–4.8%), and *Cantharellales* (0.06–3.8%) among the samples. These samples were significantly enriched in *A. mongholicus* rhizosphere soils in July and September. *Pleosporales* (6.6–91.1%) in the rhizosphere soil was significantly greater than the bulk soil. The relative abundance of *Pleosporales* (21.4–90.8%), *Thelebolales* (0.6–0.8%), and *Onygenales* (0.6–0.8%) were higher in CC6, while *Byssochlamys* was the most abundant in CC6. Specific fungal families appear similarly to the bacteria and are prevalent in different sampling periods and continuous cropping soils.

Bacterial and fungal alpha-diversity

High parametric and non-parametric diversity indices were recorded for all soils in the study (Table 2). Shannon diversity indices, Chao1, and ACE richness indices showed that the CC6 soil exhibited the highest bacterial community diversity in May and July and the lowest diversity in September. Simpson indices of the bacterial community in all soils did not vary significantly. The Shannon diversity indices of CK, CC1, and CC3 soils remained identical in September as in May, while the Chao1 and ACE richness indices were increased during growth, and were greater than the CC6 soil. The higher variabilities were seen among the CK, CC1, and CC3 soils in 16S; fingerprinting contributed to this diversity.

Diversity and richness indices of the CK soil fungal community were higher than the soils transplanted with *A. mongholicus* seedlings in July and September. The richness of the CC1 soil was significantly more than CC3 and CC6. Thus, the richness of bacterial and fungal communities were reduced in continuous cropping soil during growth.

Bacterial and fungal community structure

The result of PCA indicated that the bacterial community exhibited significant differences in May, July, and September (Fig. 4). There were large discrepancies between the May and July samples and the September samples. July and September were relatively similar, with a significantly lower principle component 1 value in the continuous cropped soil, and a higher principle component 2 value in the CK soil. The result of PCA also demonstrated variations among these differently treated soil samples with the bacterial communities in the CK soil sample separated from the other three samples. The successive cropping of *A. mongholicus* is the key factor influencing the microbial structure and diversity in the rhizosphere.

The fungal community in the soil samples from July was significantly different from those in both May and September, like the bacterial community variations among the three sampling time points. However, the fungal community showed different β- diversity in the CK, CC1, CC3, and CC6 soils at each sampling time. In July and September, the *A. mongholicus* replanted soil exhibited rather similar community members, which were remarkably different from the CK soil

by principal component 2. In addition, the fungal communities in CK and CC3 soils showed similar community members that varied from the CC1 and CC6 soils in May.

A hierarchical clustering heat map analysis was conducted at the genus level based on the top 35 most abundant bacterial and fungal communities across twelve soil samples. The analysis showed that the CC1, CC3, and CC6 soils sampled in July and September were clustered together, independently (Fig. 6). They were separated, however, from the CK soils and the results agreed with those of the PCA. In addition, *Mycobacterium, Lupinus, Hyphimicrobium*, and *Steroidobacter* genera in the CC1, CC3, and CC6 soils sampled in September were found with high Z-scores of the relative percentage. *Pseudoxanthomonas, Novosphingobium, Thermomonas, Kaistobacter,* and *Arenimonas* genera in the CC1, CC3, and CC6 soils sampled in July were found to exhibit a high Z-score. On the other hand, these genera of *Proteobacter* showed a low Z-score in the CK soil. Some differences between the bacterial genera belonging to *Planctomycetes, Proteobacteria,* and *Verrucomicrobia* phylum were observed in the CK (May) and CC3 (May) soil, despite being grouped together.

Differences between the fungal community structures were shown as a heat map (Fig. 7) and PCA (Fig. 5). The top 35 most abundant fungal genera of the CC1, CC3, and CC6 soils sampled in September were clustered together independently and separated from the soils sampled in July and May. While fungal genera of the continuously cropping soils were obviously separated from the CK soil irrespective of the stage. The highly abundant fungal genera were distributed in both treatments.

Enzyme activities of replanted *A. mongholicus* rhizosphere soils

Soil urease, cellulase, and invertase activities were assessed to measure the potential turnover rates of nitrogen or carbon in the replanted *A. mongholicus* soils. These enzyme activities were also assumed to be related to the total number of the soil microbes. Soil urease activity deceased with the continuous cropping of *A. mongholicus* (Table 3). Cellulase activity showed a trend converse to the urease activity in which CC6 soil displayed the highest activity. Soil invertase activity in the CC1 soil was significantly higher than the CC6 soil.

Discussion

Soil is one of the most difficult environments to investigate due to its complexity, leading to additional methodological challenges from soil sampling to sequencing analysis (*Lombard et al., 2011*; *Lupatini et al., 2013*). In this study, we assessed the dynamics of the relative abundance and community structure of selected soil microbial communities as a function of continuous cropping and the sampling stage, using *A. mongholicus* seedlings. The effects of continuous cropping and the sampling stage on plant-associated microbial communities were measured by pyrosequencing techniques of extracted DNA (i.e. 16S rRNA genes for bacteria and ITS genes for fungi).

Continuous cropping influenced soil microbial community structure

The rhizosphere, as nutrient rich niches, are well suited for copiotroph (r-strategists) such as several types of *Proteobacteria*. *Proteobacteria* have been classified as “copiotrophs” and grow rapidly in a nutrient-rich environment, which encompasses an elevated level of morphological,

physiological, and metabolic diversity and play significant roles in global nutrient cycling (*Li et al.,2018*; *Kersters et al., 2006*). The relative abundance of *Alpha, Beta,* and *Gamma Proteobacteria* have been reported to be significantly higher in legume-based treatments (*Trivedi et al., 2015*). *A. mongholicus* is a leguminous plant, and hence, the higher relative abundance of *Proteobacteria* in the rhizosphere soil than in the bulk soil at the September time point is not surprising. Continuous cropping results in the depletion of soil nutrients. *Actinobacteria* and *Acidobacteria* are classified as "oligotrophs" (k-strategists) that degrade relatively recalcitrant forms of C, grow slowly, and are dominant in nutrient-poor environments (*Fierer et al., 2007*; *Trivedi et al., 2013*). These bacteria were at lower relative abundances in the *A. mongholicus* rhizosphere soil at the September time point, and decreased with the continuous cropping years. The phylum *Acidobacteria* has been reported as predominant in soil (*Gottel et al., 2011*; *Xiong et al., 2015*), which is in agreement with our findings.

The variation in the bacterial community of the continuously cropped soil was complex. At the order level, *Rhizobiales, Sphingomonadales, Burkholderiales, Rhodocyclales,* and *Xanthomonadales* were predominant in the *A. mongholicus* rhizosphere and bulk soil. Among these, *Rhizobiales, Burkholderiales, Xanthomonadales*, *and Myxococcales* showed a successively increasing presence with continuous cropping in September and May. These bacteria are also reported to be associated with peanut (*Arachis hypogaea L.*) continuous cropping (*Chen et al., 2014*). *Rhizobiales* and *Burkholderiales* were the most dominant root nodule-forming bacteria associated to legumes (*Postma et al., 2016*). The root nodule bacteria fix the atmospheric dinitrogen making it available for the plant in exchange for organic carbon compounds (*Moulin et al., 2001*). This mutualistic interaction might be critical for both plants and bacteria. Some bacteria pathogens of *Burkholderiales* may be associated with the increasing tendency of the continuous cropping obstacle. *Actinomycetales, Sphingomonadales, Rhodobacterales,* and *Solirubrobacterales* have greater relative abundances in continuous cropping after 6 years compared with 1 year, while lower than 3 years. Among these, *Solirubrobacterales* has been reported to be positively correlated with apple plant growth (*Franke-Whittle et al., 2015*) and negatively correlated with peach shoot weight (*Yang et al., 2012*), thereby demonstrating that *Solirubrobacterales* can variably affect plant growth, however, the mechanism of this effect is uncertain. The *iii1-15* is a dominant order of *Acidobacteria* widely distributed in each treatment and each sampling time point. The bacteria in the phylum *Acidobacteria* were reported at higher frequencies in the rhizosphere of healthy plants than the diseased plants (*Yin et al., 2013*). This might explain the relative abundance of *iii1-15* with continuous cropping.

Further analysis of the genera showed significant differences among treatments with the relative abundances of *Pseudomonas, Lysobacter,* and *Phenylobacterium* in the continuously cropped 6 year soil being higher than other treatments. Moreover, comparable results were also found in apple nurseries, suggesting that the higher percentage of *Pseudomonas* was related to the replant disease (*Sun et al., 2014*). The increase of *Lysobacter* was possibly induced by the high percentages of pathogenic fungi. *Pseudomonas* has been proposed to play a role in

replanting disease etiology of peach and apple trees (*Rumberger et al., 2007*) through the production of hydrogen cyanide (HCN). It is also considered to be a major group of rhizobacteria that may harbor the potential to regulate the physiology of plant pathogens (*Pliego et al., 2011*) and cause negative effects due to continuous cropping. *Lysobacter* has been proposed as a rich source of novel antibiotics with which some species may potentially regulate plant diseases (*Xie et al., 2012*).

Fungal coverage values of 99% were obtained for all soils by pyrosequencing, which indicates that the diversity in the soils was adequately investigated. We found that the largest relative abundance of fungi in the rhizosphere soil was *Ascomycota*, followed by *Basidiomycota, Zygomycota, Chytridiomycota,* and *Glomeromycota*, which suggested a ubiquity of these species and a key role in agroecosystems. Moreover, the relative abundance of *Ascomycetes* in rhizosphere soil was significantly higher than that in the bulk soil and showed increased relative abundances in the 6-year continuous cropping soil. After 6 years of continuous cropping, the relative abundance of *Pezizomycetes* and *Eurotiomycetes* displayed pathogenic fungi in the subordinate categories. These findings support that soil fungal communities are closely linked with the continuous cropping of the *A. Mongholicus* years. Further analysis of the genera showed that *Erysiphe, Phaeomycocentrospora, Fusarium, Geomyces, Rhizophlyctis,* and *Volvariella* constituted the predominant genera; among these *Erysiphe and Fusarium* were significantly different in various processing. The appearance of these species could be responsible for the continuous cropping. For example, *Erysiphe* was found to be a pathogen in the *Arabidopsis* powdery mildew (*Koh et al., 2005*). In this study, enriched *Erysiphe* was detected in the rhizosphere soil in July. Concurrently, we observed white patches on the leaves in the *A. mongholicus* soil that were suspected to be mildew, suggesting a link between *Erysiphe* and the growth of *A. mongholicus*. *Fusarium* is typically considered to be a member of the root rot of several types of plants as it is the most frequently occurring genus among root-colonizing fungi (*Tewoldemedhin et al., 2011*). *Fusarium* was abundant in the continuously cropped 6 years soil sampled in May, and there was a decreasing trend noted in the relative abundance of *Fusarium* during the growth period. However, the relative abundance was as high as others in July and September, albeit without a significant difference with other continuous cropping systems. This phenomenon could have transferred to, caused the death of, or infected the plants, the root rot of *A. mongholicus* was severe in July and September, suggesting that the *Fusarium* species inhabiting the rhizosphere can have a relevant negative role on plant growth. In addition, the unidentified groups may play a key role in the continuous cropping soils. Further investigations are required to identify their functions associated with *A. mongholicus* cropping.

Heat map analyses and PCA demonstrated dynamic changes in the bacterial and fungal populations of the soil owing to the continuous cropping of *A. mongholicus* and the sampling stage. These results demonstrated that the soil microbial communities were affected by the continuous cropping of *A. mongholicus*. The soil microbial biomass and structure were also significantly influenced by continuous cropping in the cases of cucumber, maize, rice, and peas (*Yao et al., 2006*; *Nayyar et al., 2009*; *Kiharaa et al., 2012*). These findings indicated that

successive changes in the soil microbial community structure with the continuous cropping system caused an aversion to the growth of *A. mongholicus*. This reveals that some of the microbes and pathogens participating in the nutrient cycle of the soil increased while the beneficial microbes decreased, which consequently caused serious continuous cropping problems in the replant system. These results further confirmed that replanting can promote disease through changes in the root-associated microbial communities.

Sampling stage influenced soil microbial community structure

The microbial community of the soil is known to be influenced by a wide range of factors such as soil characteristics, environmental conditions, time, and crop management strategies, such as rotations and the removal of crop residue (*Govaerts et al., 2007*; *Mclaughlin et al., 1995*). Differences in the microbial community in bulk soil between the pre-planting and young-plant stages have been shown previously (*Larkin et al., 2003*). Therefore, in this study, we assessed the soil bacterial and fungal dynamic succession at different sampling stages of different *A. mongholicus* continuous cropping systems, including pre-planting (May), the rapid growth phase (July), and the harvest stage (September), to provide general data about the relationships between the soil microbial community and the continuously cropped *A. mongholicus.* The diversity and richness of bacteria in the rhizosphere soil cropped continuously for 6 years to *A. mongholicus* at the pre-planting stage were higher than at the rapid growth, and harvest stages, which was contrary to the richness of the bacterial population in the *A. mongholicus* rhizosphere soil replanted at 1 and 3 years. The diversity and richness of fungi in the rhizosphere soil were decreased during the *A. mongholicus* growth. The soil from the 6 years of continuous cropping was distinct from other soils. These results showed that the diversity of the bacterial and fungal communities were reduced during growth, especially in the 6 year continuously cropped *A. mongholicus* rhizosphere soil. Conversely, the key factor in explaining the variation of the microbial community structure in the soil was the stage of plant growth (*Wang et al., 2016*). The average relative abundance of *Proteobacteria* and *Acidobacteriales* in the harvest stages was higher than at the pre-planting stage, whereas, *Actinobacteria* and *Basidiomycota* were reduced during growth. The relative abundance of *Cyanobacteria* increased significantly at the harvest stages of *A. mongholicus* which may be related to its nitrogen fixation and environmental resistance. In addition, at the pre-planting stage *Pseudomonadales, Bacillales, Rhodobacterales, Ustilaginales,* and *Thelebolales* orders were specifically enriched in the replanted *A. mongholicus* rhizosphere soil (both of CC1, CC3, CC6) than the growth phase, while *Xanthomonadales, Myxococcales, Acidimicrobiales* were specifically enriched at the rapid growth phase, and *Rhodocyclales* and *Agaricales* were enriched at the harvest stages. Based on the analyses, these populations may be related to the growth and development of *A. mongholicus.* Hierarchical clustering analysis showed that bacterial and fungal genera clustered together at the rapid growth phase and harvest stages. Compared with the continuous cropping years, the microbial community structure varied greatly at different sampling periods, which might be explained by the root exudate content in the *A. mongholicus* rhizosphere that changes with the growth period. For instance, flavonoids can attract specific microorganisms (*Bennett et al., 2007*)

and are a major component of *A. mongholicus* (*Li et al., 2014*). The effect of the sampling season on the *Cyclopia spp* rhizosphere bacterial communities may be attributed to the soil temperature and moisture, as well as the alteration of root exudates between seasonal growth and the developmental phases (*Postma et al., 2016*). Interestingly, the eukaryotic microorganisms did not show any obvious correlation with peanut development in an earlier study (*Chen et al., 2012*). Thus, the mechanisms underlying the interaction between microbial communities and the growth or development of *A. mongholicus* need to be investigated further.

Continuous cropping influenced the soil enzyme activities

Replanted soil significantly inhibited root development and exhibited different soil enzyme activity. The soil enzyme activities are considered to be vital indicators of soil quality and ecological stability, and thus, crucial indicators of the soil biochemistry (*Gianfreda et al., 2005*). Knowing the characteristics of soil enzyme activities in *A. mongholicus* continuous cropping systems could be beneficial in providing a better understanding of soil productivity in replanted *A. mongholicus*. Urease catalyzes the hydrolysis of urea to produce ammonia and carbamate, and thus, is recognized as a critical indicator of soil health. In this study, the rhizosphere soil of *A. mongholicus* showed significantly decreased activities after 6 years of continuous cropping. These results were consistent with the previous study that continuous monocropping of peanut and black pepper led to a decrease in soil urease and invertase activities (*Xiong et al., 2015*). These results indicate that the root exudates in new planting soil might support a new and different functional microbial community that might be responsible for the apparent increase in mineralization, resulting in an enhanced supply of available nutrients as compared to continuous cropping soils. Additionally, after many years of continuous cropping, the root system absorbed the available nutrients resulting lower activities of the rhizosphere soil urease than in the bulk soil. Sun et al. reported that urease was highly correlated to the apple tree growth (*Sun et al., 2014*). The lack of a significant increase in urease activity at the replant site might explain the decreased growth, and *Rhizobiales* and *Burkholderiales* showed an increasing abundance under continuous cropping. However, soil invertase and cellulase promoted the conversion of carbohydrates and participated in organic matter cycling in the soil (*Han et al., 2012*; *Paz-Ferreiro et al., 2014*). The invertase affects the hydrolysis of sucrose into glucose and fructose, and cellulase is involved in breaking down cellulose. The rhizosphere soil invertase activities significantly decreased with replanted *A. mongholicus*, which was contradictory to the actions of cellulase, both were higher than the bulk soil. This could be related to the residual small root tissues of previous *A. mongholicus*. The results revealed a significant decline in enzymatic activities under the long-term continuous cropping system, which might limit *A. mongholicus* growth.

Conclusions

In this study, the succession dynamics of the microbial communities within the soil with the continuous cropping of the economic medicinal plant *A. mongholicus* has been comprehensively analyzed. A major effect of sampling stages, as well as continuous cropping years, was noted on the microbial communities associated with *A. mongholicus.* The variations can be summarized as

five major highlights: (1) In September, continuous cropping for 6 years of the *A. mongholicus* rhizosphere soil (CC6) presented a lower diversity and relative abundance than the *A. mongholicus* rhizosphere soil without continuous cropping (CC1), (2) from May to September, the relative abundance of *Fusarium* displayed a steady decline in CC6, while it was significantly greater than CC1 and CC3 in May, (3) *Erysiphe* was enriched in July and this could be related to the diseases of *A. mongholicus*. In the 6 years of continuous cropping, the relative abundance of *Rhizobiales, Sphingomonadales, Burkholderiales, Rhodocyclales, Xanthomonadales* was higher than in CC1 and CC3 soils. (4) The relative abundances of *Actinomycetales, Sphingomonadales, Rhodobacterales,* and *Solirubrobacterales* were lower in the 6 than 3 years of continuous cropping in September, (5) Significant decline in urease and invertase activities were observed after 6 years of continuous cropping. In conclusion, the findings of this study, investigating both bacterial and fungal communities in parallel, demonstrate the advantages of deep-throughput sequencing in elucidating the biotic components of disease complexes which affect continuous cropping *A. mongholicus*.

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Table 1(on next page)

A number of effective tags and OTUs of *A. mongholicus* rhizosphere soil bacteria and fungi.

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Values are the means ± SE (n = 3).

Table 1. A number of effective tags and OTUs of *A. mongholicus* rhizosphere soil bacteria and fungi.

Soil	May		July		September	
	Effective Tags	OTUs	Effective Tags	OTUs	Effective Tags	OTUs
Bacteria						
CK	78008±22702 ab	4744±479 cd	55014±8437 b	4293±250 d	87361±23039 ab	5396±595 abcd
CC1	75368±16511 ab	5109±463 bcd	57609±8067 b	4471±332 cd	97286±22126 ab	6004±508 ab
CC3	94721±18141 ab	5518±398 abc	54015±4092 b	4385±158 cd	75370±47383 ab	4685±1176 cd
CC6	107505±3686 a	6158±193 a	58402±7298 b	4695±145 cd	49456±22887 b	4387±512 cd
Fungi						
CK	57812±5012 ab	535±51 b	53994±10347 ab	882±59 a	41683±16645 ab	656±185 ab
CC1	36163±12296 b	722±153 ab	50305±9715 ab	695±77 ab	48189±5312 ab	317±72 c
CC3	70841±25345 a	546±51 b	43551±11742 ab	624±196 ab	49566±7689 ab	288±58 c
CC6	53386±22888 ab	887±267 a	52109±10979 ab	629±108 ab	52597±6052 ab	279±29 c

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Samples followed with M, J or S represent soil sampled in May, July or September.

Table 2(on next page)

Bacterial and fungal α-diversity indexes of *A. mongholicus* rhizosphere soil. The numbers are averages of four replicates from each treatment and sampling time point.

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Values are the means ± SE (n = 3).

Table 2. Bacterial and fungal α-diversity indexes of *A. mongholicus* rhizosphere soil. The numbers are averages of four replicates from each treatment and sampling time point.

Soil	May					July					September				
	Shannon	Simpson	Chao1	ACE	Coverage (%)	Shannon	Simpson	Chao1	ACE	Coverage (%)	Shannon	Simpson	Chao1	ACE	Coverage (%)
Bacteria															
CK	9.74±0.08 c	0.996 a	4169 a	4421±291 bc	94.5±0.005 a	9.98±0.04 abc	0.997 a	4307 a	4481±80 bc	94.4±0.002 a	10.06±0.06 abc	0.997 a	4567 a	4794±409 abc	94.0±0.006 ab
CC1	10.07±0.08 abc	0.998 a	4564 a	4783±248 abc	94.0±0.004 ab	9.99±0.11 abc	0.998 a	4278 a	4512±565 bc	94.4±0.009 a	10.15±0.21 ab	0.997 a	5098 a	5306±540 ab	93.2±0.008 ab
CC3	9.76±0.10 c	0.995 a	4642 a	4825±208 abc	93.8±0.003 ab	10.00±0.06 abc	0.998 a	4277 a	4390±301 c	94.6±0.005 a	9.77±0.27 c	0.994 a	5243 a	4920±675 abc	93.7±0.009 ab
CC6	10.31±0.09 a	0.998 a	5375 a	5548±230 a	92.9±0.003 b	10.15±0.06 ab	0.998 a	4490 a	4715±342 abc	94.2±0.006 ab	9.88±0.28 bc	0.995 a	4588 a	4746±124 abc	94.0±0.002 ab
Fungi															
CK	3.75±0.16 bc	0.821 a	521 cde	538±85 cde	99.6±0.001 abc	5.61±0.05 a	0.922 a	884 ab	927±22 ab	99.2±0.001 c	4.41±0.53 abc	0.836 a	619 abcd	633±163 bcd	99.5±0.002 abc
CC1	5.29±0.68 ab	0.901 a	750 abc	771±188 abc	99.4±0.002 abc	4.25±1.03 abc	0.793 a	715 abc	739±123 abc	99.3±0.001 c	1.28±0.34 d	0.284 b	356 de	385±57 de	99.6±0.001 ab
CC3	3.50±0.21 c	0.792 a	506 cde	538±40 cde	99.5±0.001 abc	3.44±1.68 c	0.630 a	624 abcd	645±241 bcd	99.4±0.002 abc	1.48±0.88 d	0.365 b	275 e	284±37 e	99.7±0.001 a
CC6	5.79±0.30 a	0.950 a	933 a	958±309 abc	99.2±0.003 c	3.62±1.47 c	0.687 a	592 bcd	630±96 bcd	99.4±0.001 abc	1.00±0.18 d	0.222 b	264 e	289±46 e	99.7±0.001 ab

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Samples followed with M, J or S represent soil sampled in May, July or September.

Table 3(on next page)

Soil enzyme activities of different years from continuously cropped *A. mongholicus* soil.

Different letters in columns indicate the significant differences (P<0.05). Values are means ± standard deviation (n=3). CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control.Values are the means ± SE (n = 3).

Table 3. Soil enzyme activities of different years from continuously cropped *A. mongholicus* soil.

Soil	Urease activity (NH_3-N mg·g^{-1} soil)	Cellulase activity (GE mg·g^{-1} soil)	Invertase activity (glucose mg·g^{-1} soil)
CK	0.605 ± 0.023 a	0.175 ± 0.003 b	2.08 ± 0.154 c
CC1	0.577 ± 0.017 a	0.177 ± 0.009 b	3.59 ± 0.303 a
CC3	0.509 ± 0.015 b	0.187 ± 0.017 b	3.27 ± 0.297 ab
CC6	0.499 ± 0.010 b	0.282 ± 0.012 a	2.78 ± 0.271 b

Different letters in columns indicate the significant differences ($P<0.05$). Values are means ± standard deviation (n=3). CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control.

Figure 1(on next page)

A. mongholicus root biomass (A) and ratios of rot/top (B) with different continuous cropping years.

Different letters in columns indicate significant differences ($P < 0.05$). CC1, transplant of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplant of *A. mongholicus* seedlings into soil that was continuously cropped for 2 years; CC6, transplant of *A. mongholicus* seedlings into soil that was continuous cropped for 5 years.

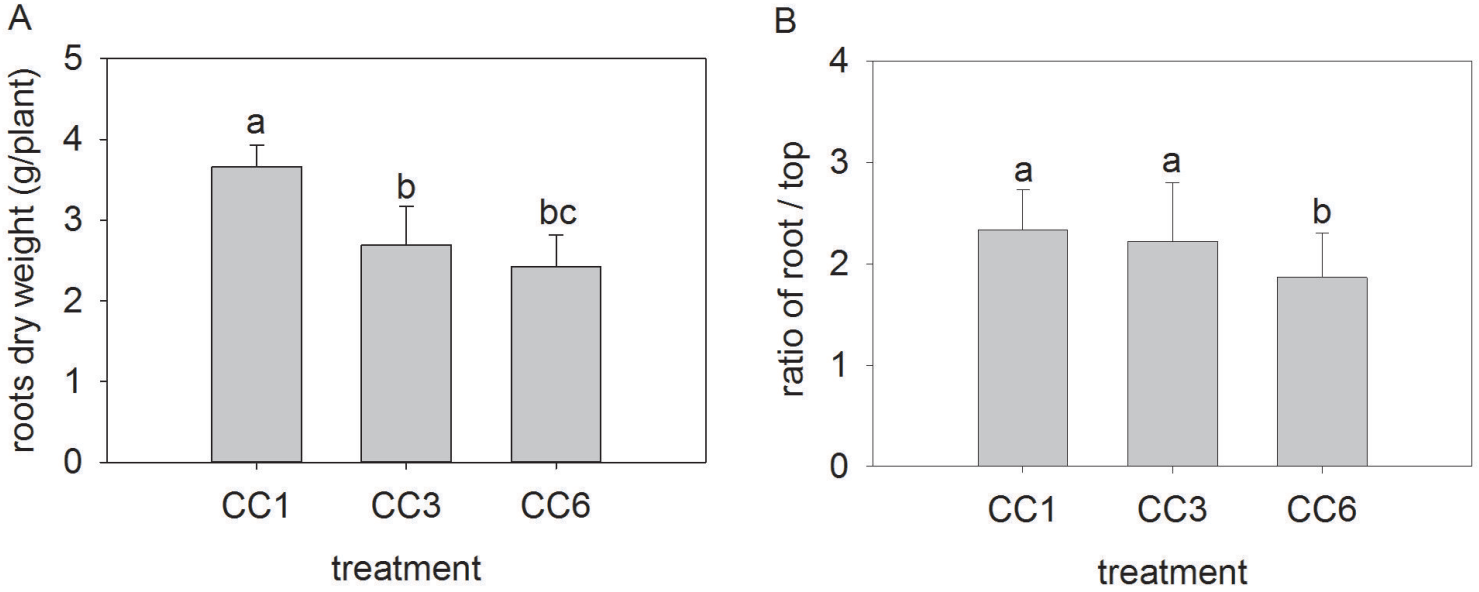

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Figure 2(on next page)

Sequencing depth of *A. mongholicus* rhizosphere soil bacteria (A) and fungi (B).

CC1, transplant of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplant of *A. mongholicus* seedlings into soil that was continuously cropped for 2 years; CC6, transplant of *A. mongholicus* seedlings into soil that was continuous cropped for 5 years. Samples followed with M, J or S represent soil sampled in May, July or September.

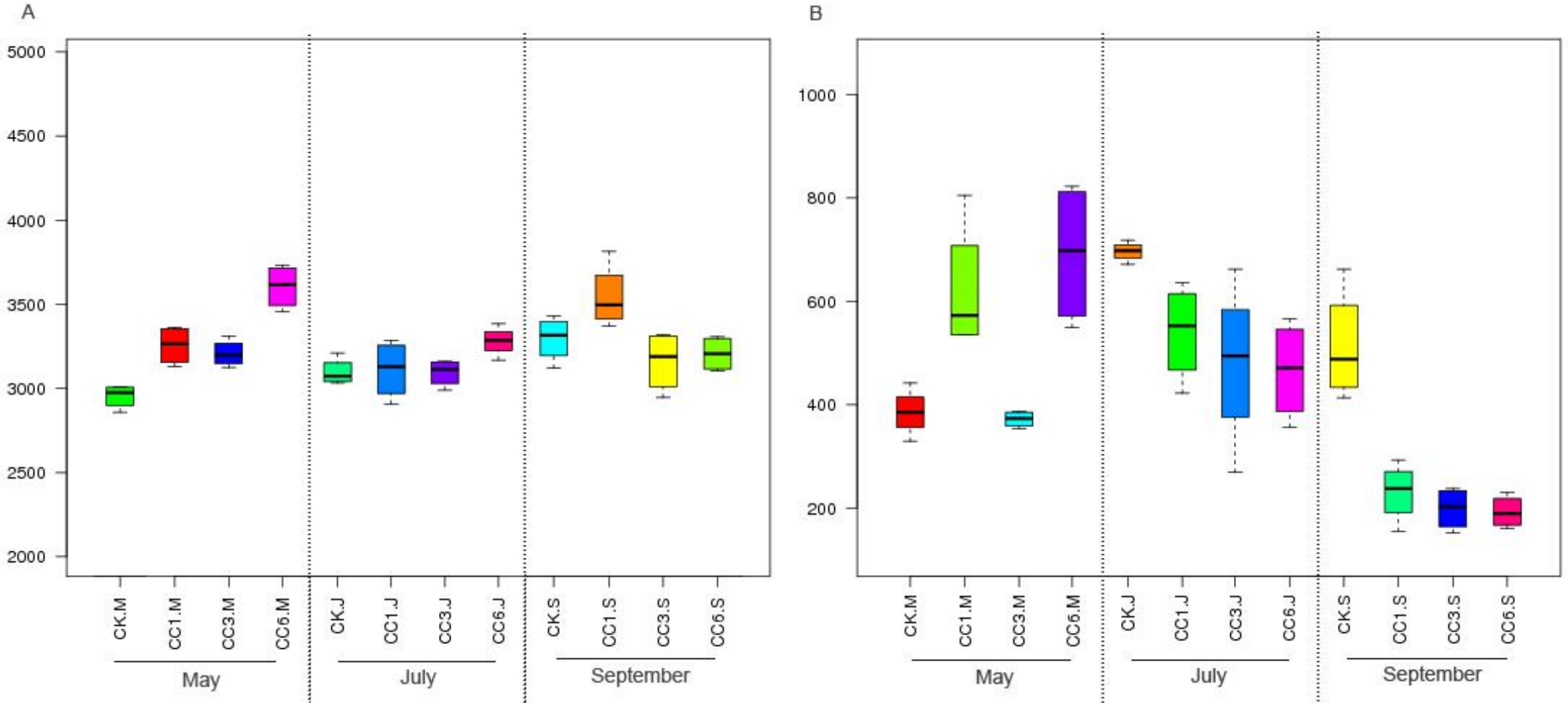

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Figure 3(on next page)

Relative abundance of the dominant bacterial phyla (A), fungal phyla (B), bacterial order (C), and fungal orders (D) in *A. mongholicu*s rhizosphere soils.

CC1, transplant of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplant of *A. mongholicus* seedlings into soil that was continuously cropped for 2 years; CC6, transplant of *A. mongholicus* seedlings into soil that was continuous cropped for 5 years. Samples followed with M, J or S represent soil sampled in May, July or September.

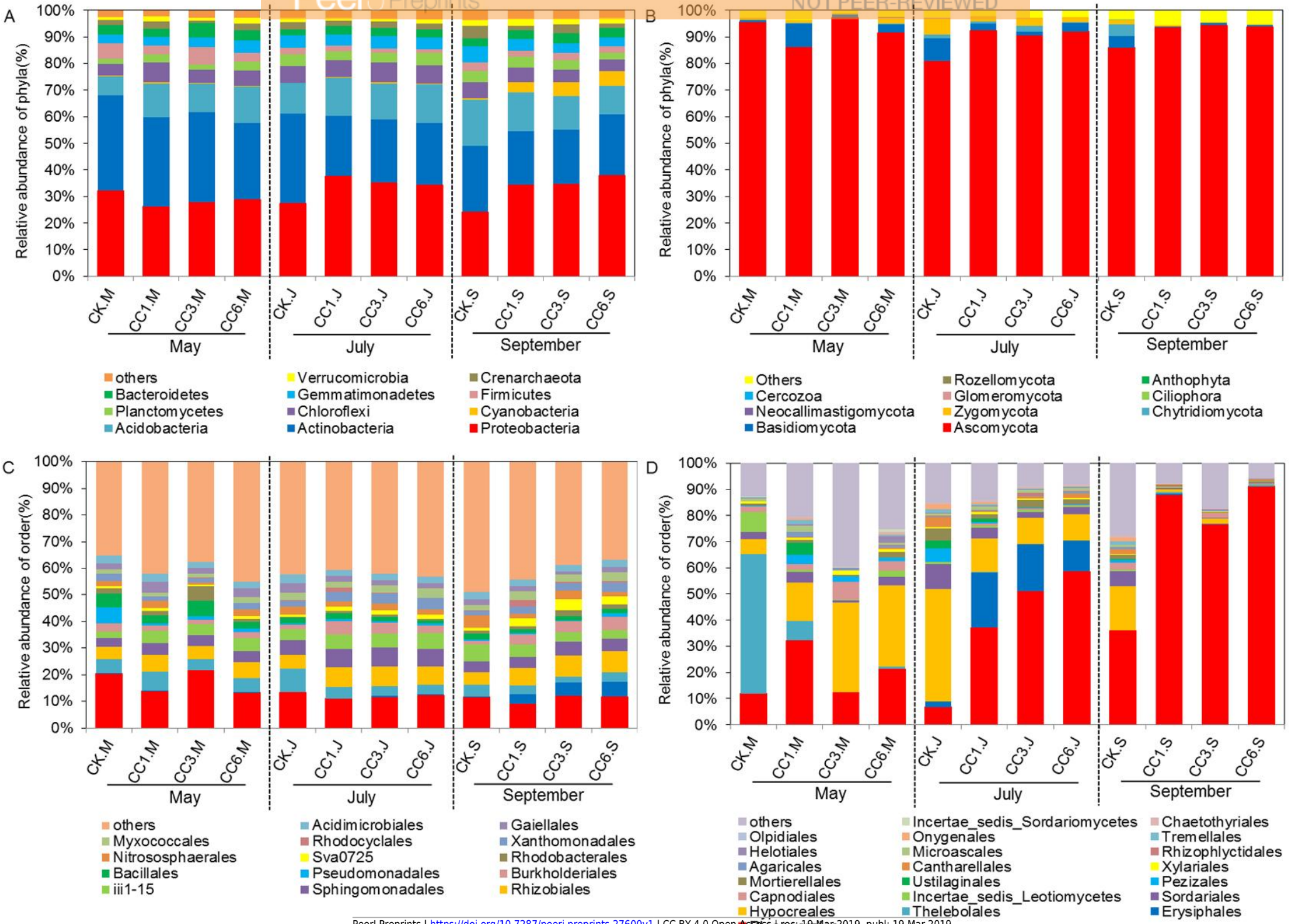

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Figure 4(on next page)

PCA of the *A. mongholicus* rhizosphere soil bacteria.

CC1, transplant of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplant of *A. mongholicus* seedlings into soil that was continuously cropped for 2 years; CC6, transplant of *A. mongholicus* seedlings into soil that was continuous cropped for 5 years. Samples followed with M, J or S represent soil sampled in May, July or September.

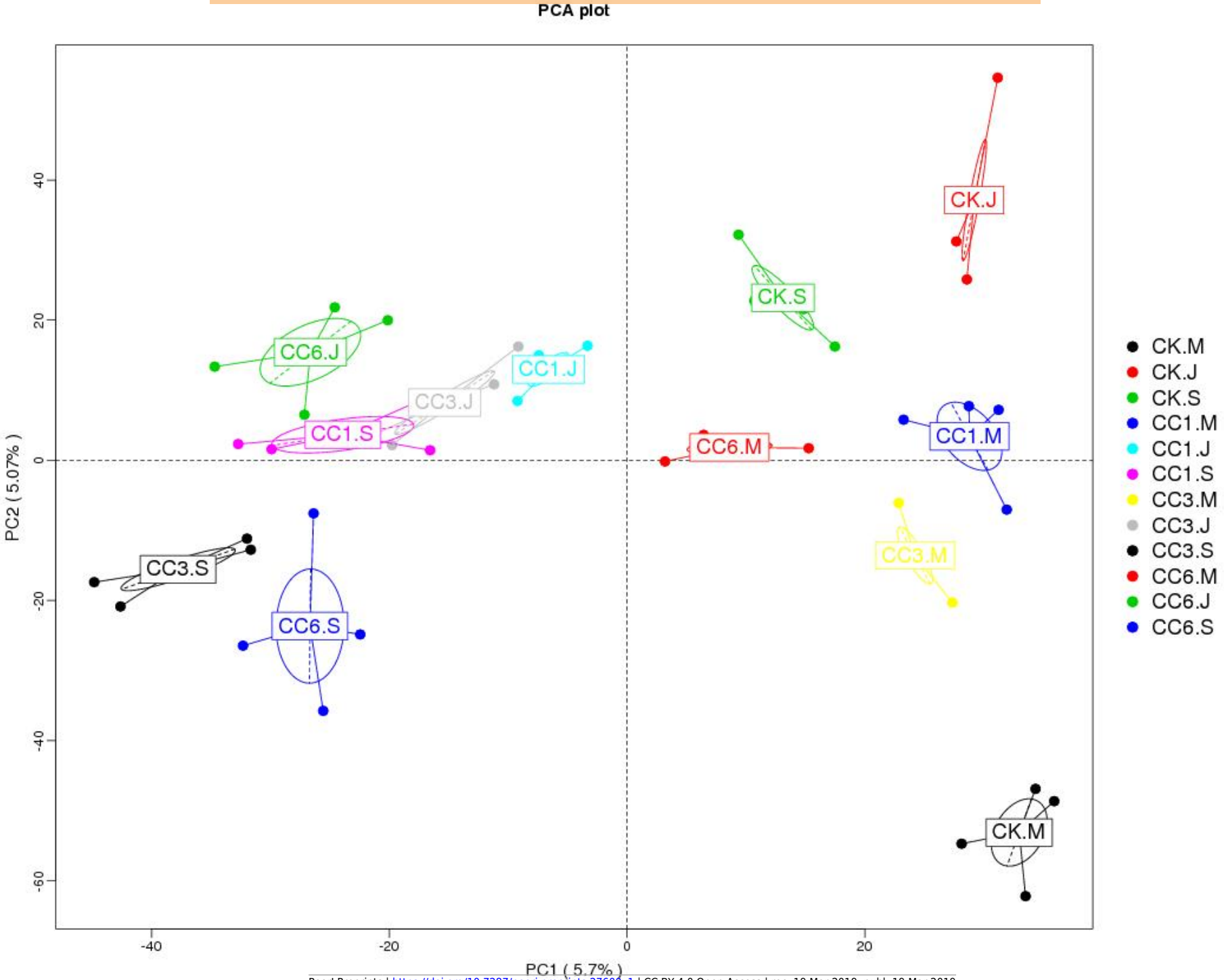

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Figure 5(on next page)

PCA of the *A. mongholicus* rhizosphere soil fungi.

CC1, transplant of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplant of *A. mongholicus* seedlings into soil that was continuously cropped for 2 years; CC6, transplant of *A. mongholicus* seedlings into soil that was continuous cropped for 5 years. Samples followed with M, J or S represent soil sampled in May, July or September.

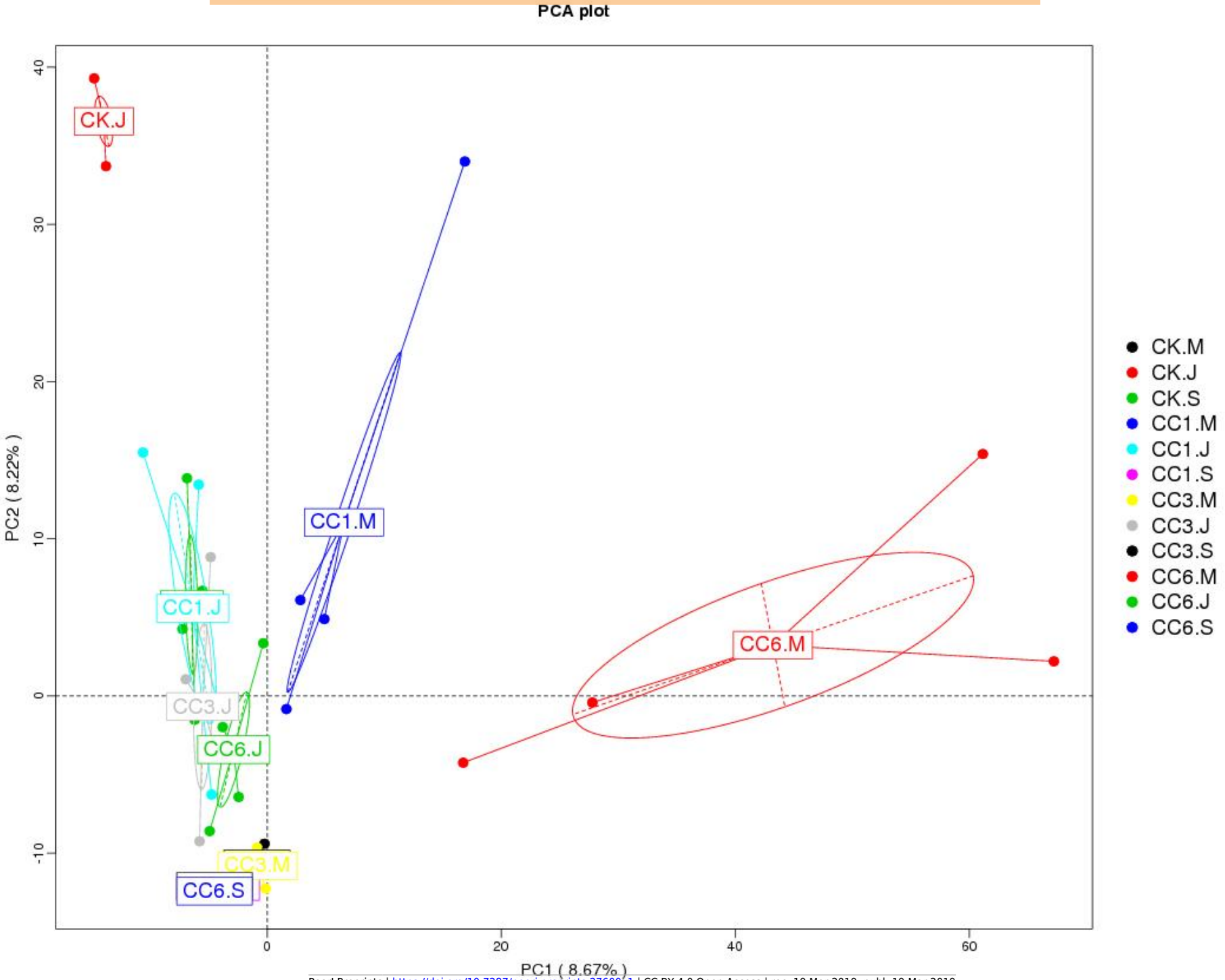

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Figure 6(on next page)

Hierarchical clustering heat map of the distribution of the dominant bacterial genera in the *A. mongholicus* rhizosphere soil.

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Samples followed with M, J or S represent soil sampled in May, July or September.

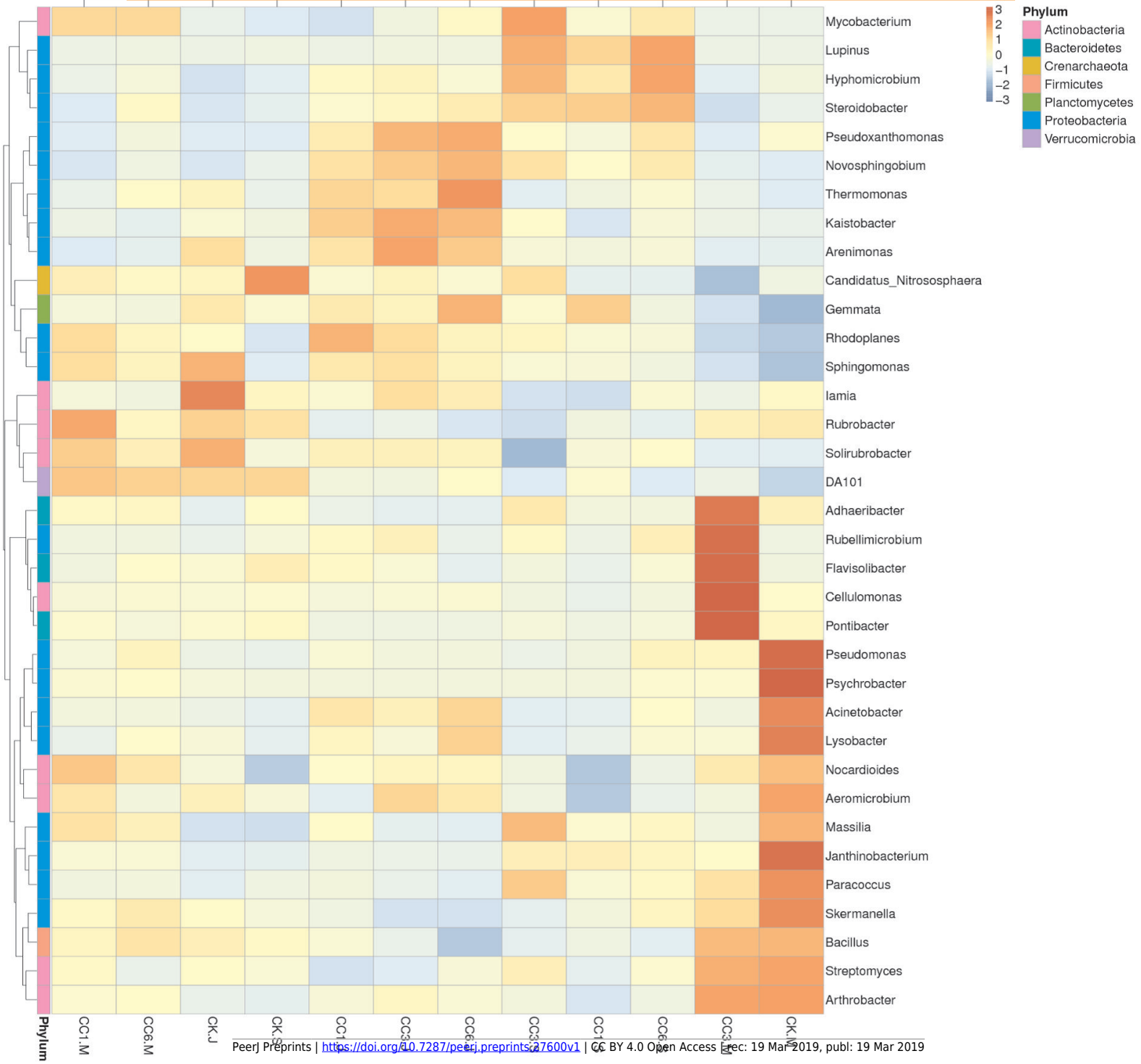
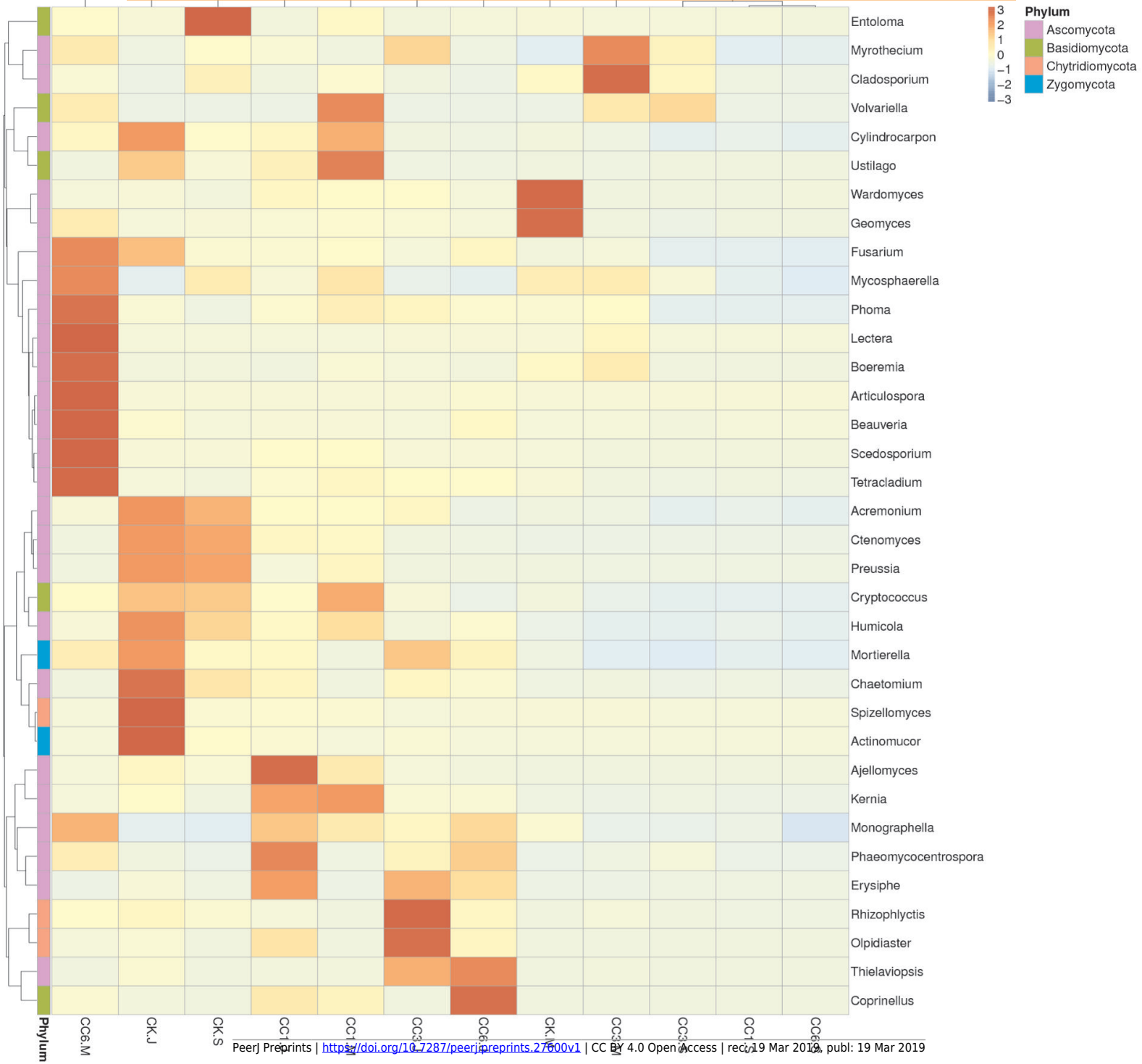


Figure 7(on next page)

Hierarchical clustering heat map of the distribution of the dominant fungal genera in the *A. mongholicus* rhizosphere soil.

CC1, transplants of *A. mongholicus* seedlings into soil that was planted with naked oats for 1 year; CC3, transplants of *A. mongholicus* seedlings into soil that was continuous cropped for 2 years to *A. mongholicus*; CC6, transplants of *A. mongholicus* seedlings into the soil that was continuously cropped for 5 years to *A. mongholicus*; and CK, the adjacent uncultivated field soil sample used as a control. Samples followed with M, J or S represent soil sampled in May, July or September.



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