Plant Soil (2022) 470:35–50
https://doi.org/10.1007/s11104-021-04913-0

REGULAR ARTICLE

Short-term lime application impacts microbial community composition and potential function in an acid black soil

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Received: 20 September 2020 / Accepted: 7 March 2021 / Published online: 14 April 2021

Abstract
Background and aims Soil acidification is a natural process that can be accelerated by intensive nitrogen fertilization. Lime application is a typical agricultural practice to enhance soil pH and increase nutrient availability for crop production. Our study aims to reveal how liming altered soil microbial community composition and potential function in the bulk and rhizosphere soils of soybean growing in an acid black soil.
Methods A short-term soybean pot experiment was conducted in an acid black soil with the amendment of five different dosages of lime. Soybean plants were harvested and soil samples were collected at the initial flowering stage. Plant biomass, shoot height and root length, as well as soil chemical properties and total microbial activities of bulk soils were measured. The abundance and composition of microbial communities in bulk and rhizosphere soils were determined using qPCR and Illumina MiSeq sequencing, respectively.
Results Liming significantly increased soybean growth and soil microbial activities, and altered soil properties such as soil pH, available phosphorus (AP), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N). Lime addition increased soil bacterial abundance and decreased fungal abundance in the bulk soils, but had no effect on microbial abundance in the rhizosphere soils as well as alpha-diversity of soil microbial community. Microbial community structures in bulk and rhizosphere soils were significantly varied with lime amendment that were related to soil chemical properties, of which soil pH was detected as the most important soil factors. In addition, liming significantly increased the potential functions of amino acid, cofactors and vitamins mechanisms of bacterial communities and the guild abundance of AM fungi.
Conclusions Lime application altered soil properties, increased soil microbial activities, and changed soil microbial compositions and potential functions, which eventually resulted in the improvement of soybean plant growth.

Keywords Bacterial community · Fungal community · Pot experiment · Soybean · · MiSeq sequencing

Responsible Editor: Xianyong Lin.

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Introduction

Soil acidification is becoming a serious threat to global terrestrial ecosystems that is responsible for the limitation factors for agricultural production (Bouwman et al. 2002; Pan et al. 2019). Acidic soils occupy approximately 30% of the world's total land areas that comprise more than 50% of potential arable lands (von Uexküll

and Mutert 1995; Kochian et al. 2015). It is noteworthy that soil acidification can be accelerated by anthropogenic activities, such as excessive application of acidifying fertilizers (Tian and Niu 2015), acidic precipitation (Lawrence et al. 2016), planting legumes (Tang et al. 1999) and crop removal (Blanco-Canqui and Lal 2009). Increasing soil acidification that can change the biogeochemistry of ecosystems and soil biology has been one of the major problems for Chinese intensive agriculture resulting from the overuse of nitrogen (N) fertilizer since the 1980s (Guo et al. 2010; Tian and Niu 2015; Zhu et al. 2018). Furthermore, continuous cropping of legumes can lead to the small area of soil acidification, which is widespread in China (Zhang et al. 2018; Bai et al. 2019; Wang et al. 2019). Therefore, more attention should be paid to the amelioration of soil acidification that is of a great significance for the sustainable development of agriculture.

Liming is a common and an effective strategy to ameliorate soil acidification for increasing soil fertility and plant productivity (Arshad et al. 1999; Deus et al. 2020). Moreover, applying lime to acid soils provides positive conditions for a series of biological processes and functional effects on ecosystem services (Nugroho et al. 2007; Liu et al. 2010a; Chatzistathis et al. 2015; Holland et al. 2018). It is reported that liming acid soils increased the rate of soil nitrogen mineralization and nitrification, and decreased the N_2O emission through slowing down the denitrification (Vazquez et al. 2019; Nadeem et al. 2020). However, previous studies were largely conducted with the long-term lime amendment, short-term liming effects were rarely reported. It is well known that soil microbial community is the crucial component of soil ecosystem and plays a key role in the ecological and physiological functions of improvement soil physicochemical conditions and soil habitability for plants (van der Heijden et al. 2008; Bardgett and van der Putten 2014). Xue et al. (2010) found that the structure, function and diversity index of soil microbial community were increased with the amount of lime addition. While Narendrula-Kotha and Nkongolo (2017) reported that lime addition had no effect on the OTU richness as well as Simpson and Shannon diversity of soil bacterial and fungal communities, but the relative abundance of *Bradyrhizobium* genus functioning as a nitrogen fixer was enriched in the limed soils using 454 pyrosequencing. Therefore, the inconsistent findings between studies highlighted that more researches are needed to analyze the abundance and diversity of soil microbial community in liming acid soils.

Black soils are one of the most important soil resources for crop production in China, which are classified as Mollisols and also named as dark Chernozems (Liu et al. 2010b). The arable black soils have been seriously degraded on account of long-term unreasonable farming practices, and soil acidification is one of the common phenomena of soil degradation in this region (Liu et al. 2010b; Zhang et al. 2020b). However, the effects of liming on the microbial communities in black soils have rarely been reported. The main objectives of this study were to explore how the composition and functional prediction of microbial community responded to the short-term of liming in an acid soil, and to evaluate the affecting factors in driving the changes of soil microbial community. In this study, a pot experiment with the application of different dosages of lime was subjected to analyze microbial community composition and potential function in the bulk and rhizosphere soils of soybean using high throughput sequencing targeting bacterial 16S rRNA and fungal internal transcribed spacer (ITS) genes. We hypothesized that (i) soil bacterial and fungal communities respond differently to lime amendment; (ii) the composition and potential function of soil microbial community are influenced by the soil properties that were influenced by liming; and (iii) the impacts of lime addition on the soil microbial community will reflect on the soybean plant growth.

Materials and methods

Pot experiment setup and sampling

A pot experiment was conducted on May 13, 2018, in the experimental garden of the Northeast Institute of Geography and Agroecology (45°70'N, 126°63′E), Harbin City, Heilongjiang Province, China. The used soil was collected from the top 20 cm of a field with soybean monoculture at Glory Village (47°21'N, 126°49′E), Hailun City, Heilongjiang Province in northeastern China. The soils (pH, 5.09) were sieved through 2 mm mesh to thoroughly homogenize and remove plant residues and stones. Applied lime was finely-ground calcitic lime (GB/T19590–2016) purchasing from Dadi calcification Co., Ltd. (Jilin, China). The soil and sand mixture in the rate of 9:1 (soil/sand; *v*/v) was used as the culture medium, which is convenient for collecting the rhizosphere soils (see Fig. S1). Six

treatments of lime addition with rate (*w*/*w*) of 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of the total soil mass were designed in this study, which coded as L0, L1, L2, L3, L4 and L5, respectively. Each treatment was performed in three repeats (pots), and 3 kg soils (culture medium) were placed in each pot (17.5 cm height and 11.0 cm diameter). After 10 days of soil pre-incubation at the soil moisture of 40%, six soybean seeds were sowed in each pot and only four soybean seedlings were kept in each pot at the stage of cotyledon extension. Subsequently, soybean plants were watered every two days with the same amount of water to keep the pot water content at 50% field capacity. At the soybean initial flowering stage (on 12 July), the plants were carefully taken out from soils by inverting the pots. Rhizosphere soil samples were collected from four plants after gentle shaking, and composited into one sample and put into a 2 ml sterile centrifuge tube. Meanwhile, the bulk soil samples were collected from each pot and sieved through a 2 mm mesh. A portion of bulk soil was collected in a 50 ml sterile centrifuge tube, the remaining soils were placed into ziplock bags (120 mm × 170 mm). The collected bulk and rhizosphere soils in the centrifuge tubes were stored at −80 °C until soil DNA extraction, the remaining bulk soils and soybean plants were temporally placed into 4 °C refrigerator.

Soil physicochemical property determination

Due to the limitation amount of rhizosphere soils, only the physiochemical properties of bulk soils were quantified. Soil pH was determined by a Thermo Orion 720 pH meter (Thermo Electron, USA) after extraction using 0.01 M $CaCl_2$ from a soil water solution (1:5, *w*/*v*) (Wang et al. 2015). Soil moisture content was measured immediately after sampling by weighing the soil before and after oven drying at 105 °C for 8 h. Other chemical properties were tested based on the methods of Lu (1999). Soil TN and TC content were determined by an elemental analyzer (Vario EL III, Germany). Soil NH_4^+-N and NO_3^--N were extracted with 2 M KCl solution (1:5, *w*/*v*), soil TP were digested with H_2SO_4-$HClO_4$, and soil AP were extracted with 0.5 M $NaHCO_3$, then the contents of soil TP, AP, NH_4^+-N, and NO_3^--N were measured using Continuous Flow Analyser (SAN++, SKALAR, Netherlands). Soil TK and AK were digested or extracted with HNO_3-$HClO_4$-HF and 1 M NH_4Ac, respectively, then assayed with inductively coupled plasma-atomic emission spectrometry (ICPS-7500, Shimadzu, Japan).

Assessment of plant growth and total soil microbial activity

Soybean plant height was measured from cotyledon scar to stem apical growth point using a tapeline, and then the plant was cut in the cotyledon scar using scissors. The soybean root was gently washed several times with water in the beaker after strictly picking out the root nodules by tweezers. Then, the root length of soybean plant was measured using Win-RHIZO Pro (version 2004a; Regent Instrument, Quebec, Canada). Finally, the fresh plant and root were enclosed in the envelops and dried at 105 °C for 30 min, then adjusted to 80 °C for 48 h and weighted to measure plant dry weight.

Total microbial activity of bulk soils was measured by the potential of fluorescein diacetate (FDA) hydrolysis (Gillian and Harry 2001). Briefly, 2 g of fresh soil (sieved <2 mm) was deposited in a 50 ml centrifuge tube with 15 ml of 60 mM potassium phosphate buffer (pH = 7.6), and 0.2 ml of FDA stock solution (1000 μg ml^{-1}) was added to start the reaction. Meanwhile, samples without adding the FDA stock solution acting as the blank control were set up. Sealing and shaking the tubes by hand and placed in the incubator at 30 °C for 20 min. At the end of incubation, 15 ml of chloroform/methanol (2:1; *v*/*v*) was immediately added into the tubes, sealing again and shaking the tubes by hand to terminate the reaction. The contents were centrifuged at 2000 rev min^{-1} for 3 min, and filtered into a new 50 ml centrifuge tube. Then, the filtrates were measured at 490 nm on a spectrophotometer using PERSEE T6-1650E (China) to show the soil microbial activity.

Soil DNA extraction

DNA was extracted from 0.5 g soil samples which kept in the −80 °C ultra-low temperature freezer by using a Fast DNA® Spin Kit for Soil (MP Biomedicals, USA). Extracted DNA was checked for quality by a NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA), and the extracted DNA was stored at −20 °C freezer until further analysis.

Quantitative real-time PCR (qPCR) and Illumina MiSeq sequencing

Absolute abundance of bacteria and fungi for all soil samples were determined by the standard curve method of quantification using LightCycler® Roche 480 with the primer sets 338F (5'-ACT CCT ACG GGA GGC AGC AG-3′) / 518R (5′-ATT ACC GCG GCT GCT GG-3′) and ITS1F (5'-CTT GGT CAT TTA GAG GAA GTA A-3′) / ITS2R (5'-GCT GCG TTC TTC ATC GAT GC-3′), respectively (White et al. 1990; Gardes and Bruns 1993). Each PCR reaction mixture was 20 μl containing 10 μl of SYBR Premix Ex Taq™ (Takara, Dalian, China), 7.0 μl sterilized ultrapure water, 1 μl of 10 μM forward and reverse primers, and 1 μl of DNA template. The qPCR program settings were as follows: initial denaturation at 95 °C for 30 s, followed by 30 cycles of denaturation at 95 °C for 5 s, annealing and elongation at 60 °C for 30 s, and one final cycle at 50 °C for 30 s for cooling. qPCRs were conducted in technical triplicates for each sample with negative controls, which contained all reagents with sterilized MilliQ water instead of soil DNA. The copy numbers of bacterial 16S rRNA and fungal ITS1 genes were calculated by the standard curves generated with 10-fold serial dilution of the plasmid containing a fragment of the 16S rRNA and fungal ITS1 genes, respectively. The data were finally normalized as gene copy number per gram of dry soil.

The V4-V5 region of the bacterial 16S rRNA gene and the ITS1 region of the fungal ITS gene was amplified using primer sets 515F (5′-GTG CCA GCM GCC GCG GTA A-3′) / 907R (5'-CCG TCA ATT CCT TTG AGT TT-3′) (Angenent et al. 2005; Brewer and Fierer 2018) and ITS1F / ITS2R modified with a unique 6 nt barcodes to differentiate soil samples, respectively. PCR reactions were conducted in technical triplicates using ABI GeneAmp® 9700 platform with 20 μl mixture consisting of 4 μl of 5 × FastPfu Buffer, 2 μl of 2.5 mM dNTPs, 0.8 μl of forward primer (5 μM), 0.8 μl of reverse primer (5 μM), 0.4 μl of FastPfu polymerase, 0.2 μl of BSA, 1 μl of DNA template and 11.8 μl of the sterile ultrapure water under the following thermal programs: initial denaturation at 95 °C for 3 min, followed by 30 cycles (95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s) for bacteria and 35 cycles (95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s) for fungi. The PCR products were pooled and purified with AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and then detected by 2% (*w*/*v*) agarose gel and quantified with Quantus™ Fluorometer (Promega, USA). The equimolar concentrations of PCR products were built libraries using NEXTFLEX Rapid DNA-Seq Kit and finally sequenced using Illumina Miseq PE 250 at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Data processing

Raw reads were processed using Quantitative Insights Into Microbial Ecology (QIIME) software (version 1.9.0) (Caporaso et al. 2010). Low quality sequences with a low average quality score (< 20) and short length (< 200 bp) were eliminated before subsequent analysis. The chimera checking and removing were performed by USEARCH software using the UCHIME algorithm (Edgar et al. 2011). The high-quality sequences were clustered to Operational Taxonomic Units (OTUs) with a 97% sequence similarity threshold based on the open reference method and the UCLUST algorithm (Edgar 2010). The taxonomic classification was conducted using the Ribosomal Database Project (RDP) classifier at an 80% confidence threshold with SILVA (Quast et al. 2013) and UNITE (Lami et al. 2020) databases for bacterial and fungal datasets, respectively. Mitochondrial and chloroplastic OTUs and singletons were removed from their final OTU data set.

Statistical analysis

To preclude bias in number of reads per sample, 23,334 and 31,606 sequences were randomly subsampled from each bacterial and fungal sample for subsequently community analysis, respectively. OTU richness, Shannon index and Faith's phylogenetic distance (PD) were calculated using QIIME software (http://qiime.org/index.html) based on *α-diversity.py*. Significant level was estimated by one-way ANOVA according to the Least Significant Difference (LSD) test ($P < 0.05$) to make a comparison among treatments using SPSS 17.0 (SPSS Inc., Chicago, USA). Canonical analysis of principal coordinates (CAP) was performed to distinguish the difference of bacterial and fungal community composition among treatments in bulk and rhizosphere soils using the *capscale* function in the R vegan packages (Oksanen et al. 2013). To solve the multicollinearity problem among soil properties, variance inflation factor (VIF) was performed using the *vif* function in the R car

packages (Fox and Wersberg 2019). Redundancy analysis and Monte Carlo permutation test were conducted to identify the significant abiotic factors that are mostly frequently related to the soil bacterial and fungal community composition using the vegan package in the R environment (R v.3.6.3) (Oksanen et al. 2013). The R Tax4Fun packages was utilized to predict potential functional profiles of bacterial community (Aßhauer et al. 2015), the OTU sequences were taxonomically classifies using BLAST search against the SILVA 123 database (Camacho et al. 2009; Quast et al. 2013) and the normalized OTU table was performed to estimate metabolic capabilities according to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway references. The functional prediction of the fungal community was conducted using the FUNGuild database with OTUs' taxonomic information (Nguyen et al. 2016). Pearson correlation analysis was conducted using R corrplot packages to illustrate the relationships among lime addition (the dosage of lime), plant growth (plant height, root length, plant dry weight), microbial activities, soil factors (soil pH, moisture, AP, NH_4^+-N and NO_3^--N), and soil microbial community diversity (Shannon index) and structure (CAP1 value).

Results

Soil properties, plant growth and soil microbial activity

Effects of lime application on bulk soil properties are summarized in Table 1. Applying lime significantly increased soil pH, and decreased soil moisture, NO_3^--N, NH_4^+-N and AP contents ($P < 0.05$). No significant differences and regular changes in other soil properties such as TN, TC, C/N, TP, TK and AK with lime amendment were observed. In addition, liming significantly increased plant height, plant dry weight and root length of soybean ($P < 0.05$) (Fig. 1a-c). Exception of treatment L1, compared with L0, the soil microbial activities were significantly increased with lime addition ($P < 0.05$), but no difference was observed among four liming dosages (L2 ~ L5) (Fig. 1d).

Soil bacterial and fungal abundances

The abundance of total bacteria and fungi in all soil samples were measured by qPCR. The mean values of the copy number of 16S rRNA from L0 to L5 in the bulk

Table 1 Effects of short-term liming on the physiochemical properties of bulk soils

Treatments[a]	Moisture (%)	pH	TC^b (g kg^{-1})	TN^b (g kg^{-1})	C/N	TP^b (g kg^{-1})	TK^b (g kg^{-1})	NH_4^+–N (mg kg^{-1})	NO_3^-–N (mg kg^{-1})	AP[c] (mg kg^{-1})	AK[c] (mg kg^{-1})
L0	12.03±1.57a[d]	5.10±0.02f	18.66±0.58a	1.61±0.04a	11.62±0.07ab	0.74±0.02a	25.29±1.87a	113.27±3.60a	3.89±0.39a	82.78±8.49a	141.80±1.59ab
L1	13.54±1.65a	5.28±0.01e	19.34±0.50a	1.62±0.04a	11.92±0.27ab	0.74±0.03a	24.21±1.03a	102.87±1.21bc	3.84±0.78ab	66.76±14.09bc	143.63±4.15ab
L2	12.53±1.40a	5.53±0.02d	18.85±1.10a	1.64±0.07a	11.49±0.16a	0.81±0.11a	24.03±0.13a	105.47±2.70b	3.84±0.2ab	55.05±7.20c	147.77±3.84a
L3	9.19±1.32b	5.90±0.07c	18.33±0.08a	1.63±0.04a	11.23±0.23b	0.85±0.19a	23.82±0.41a	105.53±0.90b	4.10±0.26a	69.82±1.30ab	141.43±6.82ab
L4	6.75±0.65c	6.71±0.05b	18.31±1.04a	1.56±0.08a	11.74±0.08a	0.69±0.02a	25.37±2.85a	103.4±1.39bc	2.97±0.63bc	33.26±1.57d	138.70±3.51b
L5	8.02±0.12bc	7.49±0.03a	18.89±0.20a	1.59±0.06a	11.88±0.38a	0.71±0.02a	23.29±0.37a	100.93±1.90c	2.32±0.25c	35.80±1.30d	143.70±4.62ab

[a] L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32%, 0.48% of total soil mass, respectively

[b] TC, TN, TP and TK represent soil total carbon, total nitrogen, total phosphorus and total potassium, respectively

[c] AP and AK represent soil available phosphorus and available potassium, respectively

[d] Different lowercase letters indicate significant difference between treatments by One-Way ANOVA ($P < 0.05$)

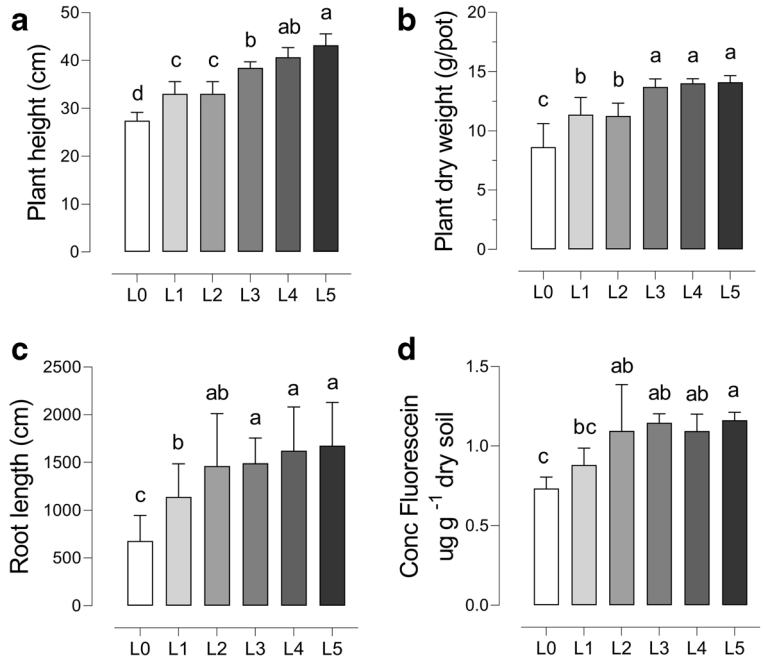

Fig. 1 Effects of liming on soybean plant height (**a**), dry weight (**b**), root length (**c**), and bulk soil microbial activities (**d**). L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively. Different letters above the columns indicate significant difference at 0.05 level

soils were 1.05×10^{11}, 1.19×10^{11}, 1.36×10^{11}, 1.55×10^{11}, 1.54×10^{11} and 1.23×10^{11} copies per gram of soil, respectively (Fig. 2a). While the mean values of the copy number of 16S rRNA from L0 to L5 in the rhizosphere soils were 2.74×10^{11}, 2.58×10^{11}, 5.39×10^{11}, 2.77×10^{11}, 3.96×10^{11} and 2.69×10^{11} copies per gram of soil, respectively. The highest copy number in the bulk and rhizosphere soils were observed in L3 and L2, respectively. For the fungal community, the mean values of copy number were 1.96×10^{9}, 1.51×10^{9}, 1.74×10^{9}, 1.83×10^{9}, 1.56×10^{9} and 1.24×10^{9} copies per gram of soil from L0 to L5 in the bulk soils, respectively (Fig. 2b). Also, 3.55×10^{9}, 3.30×10^{9}, 3.80×10^{9}, 3.26×10^{9}, 3.29×10^{9} and 3.40×10^{9} copies per gram of soil were the mean values of copy number in the rhizosphere soils from L0 to L5, respectively. Except L2 in the rhizosphere soils, the average copy number of fungi was decreased with the lime addition in the bulk and rhizosphere soils. Besides, the copy number of soil bacteria and fungi were higher in the rhizosphere soils than that in the bulk soils ($P < 0.05$) (Fig. 2).

Soil microbial community composition

The dominant bacterial phyla were Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes and Firmicutes across all samples, which was accounting for more than 85% of the total relative abundance (Fig. 3a). The lime application significantly decreased the relative abundance of Firmicutes in both bulk and rhizosphere soils, decreased the relative abundance of Cyanobacteria and Chlamydiae in the rhizosphere soil and Patescibacteria, Armatimonadetes and WS2 in the bulk soils at the high level of liming, but significantly boosted the relative abundance of Gemmatimonadetes and Rokubacteria in the bulk soils and Actinobacteria in the rhizosphere soils ($P < 0.05$) (Table S1). For the fungal communities, the dominant phyla were Ascomycota, Basidiomycota, Zygomycota and Chytridiomycota across all samples (Fig. 3b). The lime application significantly increased the relative abundance of Basidiomycota in L2 and Glomeromycota in L5 of bulk and rhizosphere soils ($P < 0.05$) (Table S2). Also, lime application in L2 and L3 significantly increased the relative abundance of Chytridiomycota in bulk soils, and significantly decreased the relative abundance of Ascomycota in rhizosphere soils except for L5 ($P < 0.05$) (Table S2).

It is noteworthy that change of soil pH resulting from lime addition was significant positively correlated with the relative abundance of Gemmatimonadetes, Bacteroidetes, Rokubacteria and Dependentiae (Fig. S2), but showed a significant negative correlation with the relative abundance of Firmicutes, Petescibacteria, Armatimonadetes, Elusimicrobia and WS2 (Fig. S2). For fungal communities, only the relative abundance

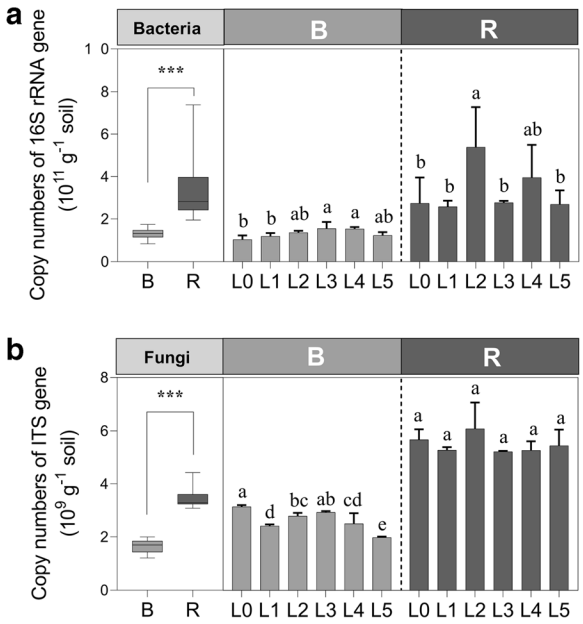

Fig. 2 Absolute abundance of bacterial 16S rRNA (**a**) and fungal ITS1 (**b**) gene copies in the bulk and rhizosphere soils of soybean growth with lime application. B: bulk soils, R: rhizosphere soils. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively. Different letters above the columns indicate significant difference at 0.05 level; three stars shows significant difference between bulk and rhizosphere soils at 0.001 level

of phylum Glomeromycota had a significantly positive correlation with soil pH (Fig. S2).

At the genus level, *Arthrobacter* and *Mortierella* were the most abundant bacterial and fungal genera, respectively (Table S3 and S4). Liming significantly increased the relative abundance of *Acidibacter*, *Phycicoccus* and *Rhodanobacer*, but significantly decreased the relative abundance of *Bacillus*, *Ralstonia, Flavisolibacter* and *Candidatus_Koribacter* in bulk and rhizosphere soils ($P < 0.05$) (Table S3). As for the fungal genera, only the relative abundances of *Monographella* and *rhizophlyctis* were significantly increased in both bulk and rhizosphere soils with lime addition ($P < 0.05$) (Table S4).

Soil microbial diversity

A total of 1,842,628 bacterial quality sequences and 2,471,501 fungal quality sequences were obtained from 36 soil samples, and these sequences were clustered into 8145 bacterial OTUs and 2721 fungal OTUs. Lime addition had little effect on the OTU richness and phylogenetic diversity of the bacterial and fungal communities (Fig. 4). However, the OTU richness and phylogenetic diversity of the bacterial communities in the bulk soils were significantly higher than those in the rhizosphere soils ($P < 0.05$), and this difference was not found in the fungal communities between bulk and rhizosphere soils (Fig. 4).

Canonical analysis of principal coordinates was used to quantify the influence of liming on the β-diversity of soil microbial communities in both bulk and rhizosphere soils (Fig. 5). The bacterial and fungal community structures were significantly different among different liming treatments in the bulk and rhizosphere soils, respectively, and the microbial communities in the rhizosphere soils showed more variance than these in the bulk soils. Besides, soil bacterial community composition showed the similar succession trend from L0 to L5 in the bulk and rhizosphere soils, but the opposite was true for fungal community.

The RDA analysis showed that soil bacterial community structure was significantly influenced by several soil factors, such as pH, AP, NO_3^--N, NH_4^+-N and soil moisture, of which soil pH was determined as the major factor (Fig. 6a, Table S5). For the fungal community structure, only soil pH was determined significantly correlated with their changes (Fig. 6b, Table S5).

Relationships among soil properties, plant growth and soil microbial community

Lime addition had significantly positive correlations with soil pH, plant height, plant dry weight, root length, soil microbial activity, and had negative correlations with soil NO_3^--N, NH_4^+-N, AP contents and soil moisture (Fig. S3). Soil bacterial and fungal β-diversity, characterized by the value of CAP1, were positively correlated with soil pH, plant height, root length, plant dry weight, and soil microbial activity, but negatively correlated with AP, NO_3^--N, NH_4^+-N and soil moisture. Moreover, soil microbial activity had a significant correlation with plant growth such as plant height, plant dry weight and root length.

Functional profiles of bacterial and fungal communities

Tax4fun was used to predict the potential functional profiles of soil bacterial community. Amino acid metabolism and carbohydrate metabolism were the two most abundant functional pathways of bacterial communities in the bulk and rhizosphere soils (Fig. 7a and

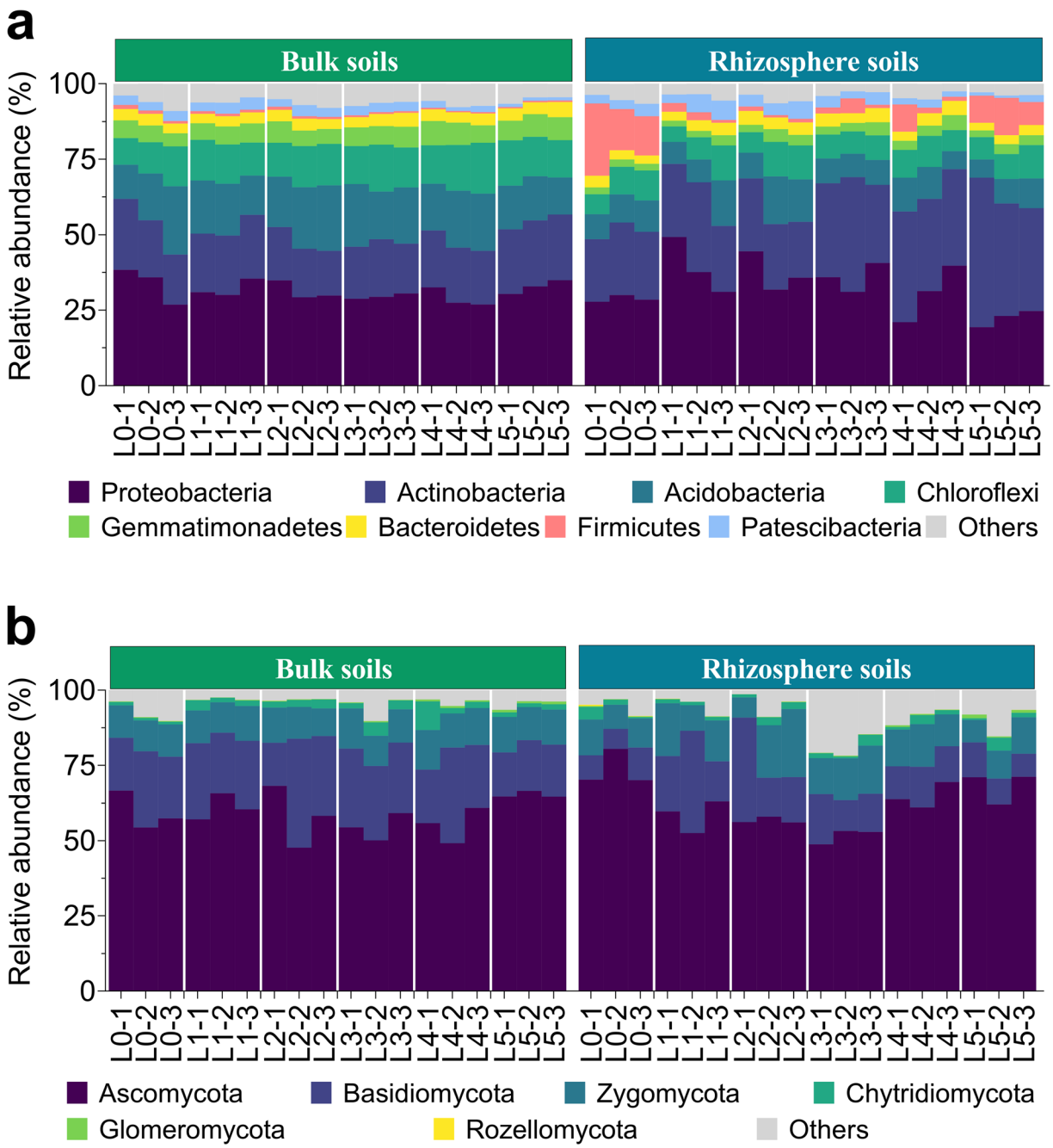

Fig. 3 Relative abundance of the major phyla of bacterial (**a**) and fungal (**b**) communities in the bulk and rhizosphere soils. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively. Number 1, 2 and 3 represent three replicates for each treatment

b). Lime application significantly increased the relative abundance of amino acid metabolism, metabolism of cofactors and vitamins and biosynthesis of other secondary metabolites in the bulk and rhizosphere soils as well as nucleotide metabolism in the bulk soils with a few exceptions ($P < 0.05$) (Fig. 7a, Table S6). Metabolism of other amino acids and glycan biosynthesis and metabolism were only significantly enriched in L1 and L4 in the bulk soils, respectively ($P < 0.05$). Also, functional pathway related to

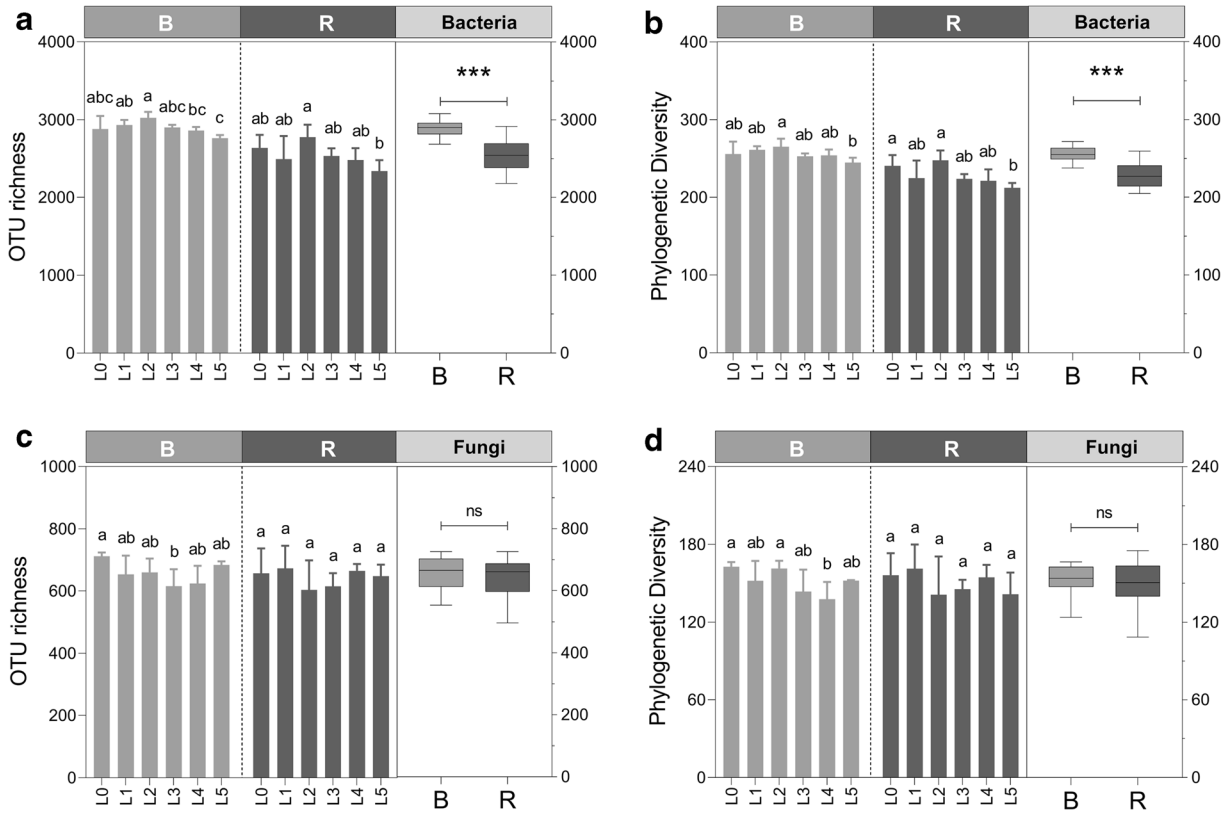

Fig. 4 The OTU richness and phylogenetic diversity of bacterial and fungal communities in the bulk and rhizosphere soils. **a** OTU richness of bacterial community; **b** Phylogenetic diversity of bacterial community; **c** OTU richness of fungal community; **d** Phylogenetic diversity of fungal community. B: bulk soils, R: rhizosphere soils. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively; Different letters above the columns indicate significant difference at 0.05 level; three stars shows significant difference between bulk and rhizosphere soils at 0.001 level; ns means no significant difference between bulk and rhizosphere soils

metabolism of terpenoids and polyketides was significantly decreased in the bulk soils ($P < 0.05$). In the rhizosphere soils, the relative abundances of energy metabolism in L2, xenobiotics biodegradation and metabolism, metabolism of terpenoids and polyketides in L5 and lipid metabolism in L3 were significantly increased with lime addition ($P < 0.05$). In addition, lime application significantly decreased the abundance of functional categories related to the nucleotide metabolism and glycan biosynthesis and metabolism ($P < 0.05$) (Fig. 7b, Table S6). Furthermore, a few metabolisms of terpenoids and polyketides pathways such as nonribosomal peptide structures (ko01054), biosynthesis of siderophore group nonribosomal (ko01053), biosynthesis of ansamycins (ko01051), biosynthesis of 12-, 14-, and 16-membered macrolides (ko00522), sesquiterpenoid and triterpenoid biosynthesis (ko00909) and biosynthesis of vancomycin group antibiotics (ko01055) were significantly decreased with the amendment of lime in the bulk soils as well as sulfur metabolism and carbon fixation in photosynthetic organisms of energy metabolism in the rhizosphere soils ($P < 0.05$) (Table S7). The lime application also enriched various metabolism pathways such as glycine, serine and threonine metabolism (ko00260), phenylalanine, tyrosine and tryptophan biosynthesis (ko00400), phenylalanine metabolism (ko00360), nicotinate and nicotinamide c metabolism (ko00760), one carbon pool by folate (ko00670), riboflavin metabolism (ko00830), taurine and hypotaurine metabolism (ko00430) across all samples. Besides, carbohydrate metabolism such as starch and sucrose metabolism (ko00500) and citrate cycle (TCA cycle) (ko00020) and fatty acid biosynthesis (ko00061) were only significantly enriched in the

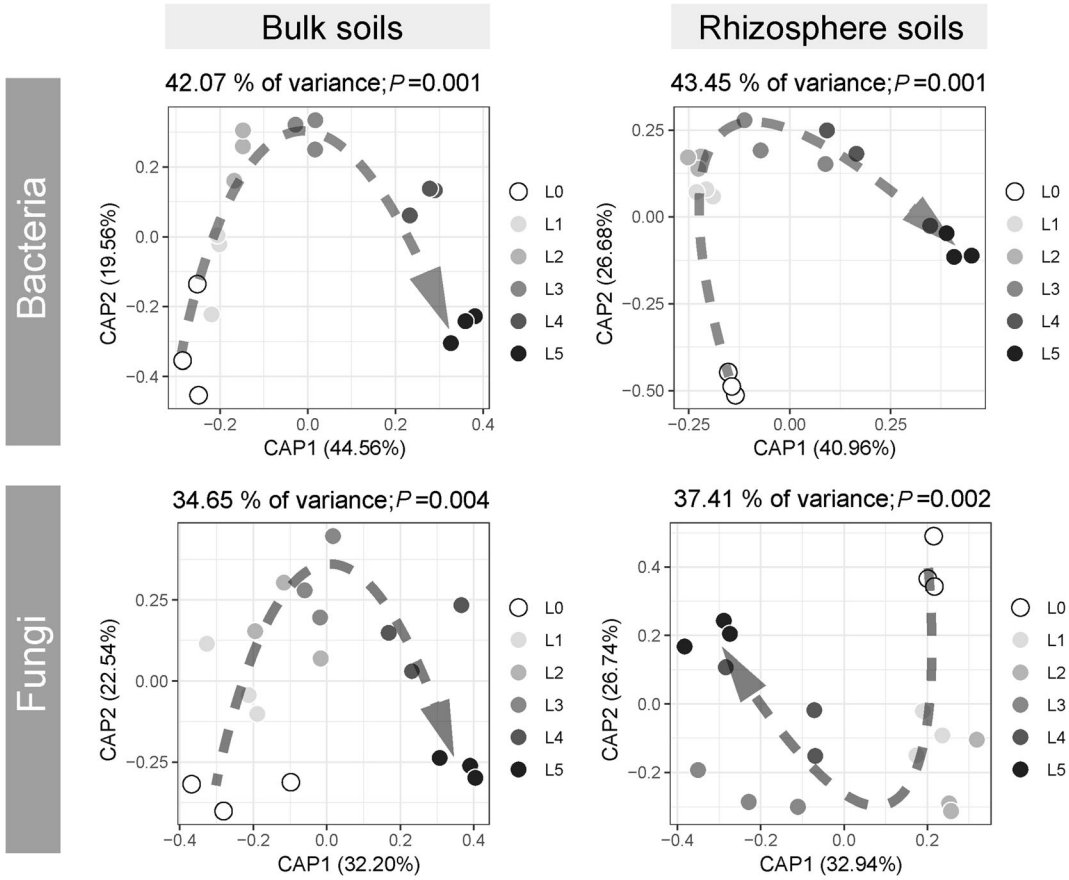

Fig. 5 Constrained principal coordinate analysis (CAP) of bacterial and fungal communities in the bulk and rhizosphere soils. The arrows show the change tendency from treatments L0 to L5. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively

rhizosphere soils with a high level of lime ($P < 0.05$) (Table S7).

FUNGuild was subjected to predict the potential functions of the fungal communities. More than 60% of the OTUs were assigned to the guilds across all samples. The undefined saprotroph was the most abundant guild in the bulk soils, followed by the animal pathogen that had higher guild abundance in the

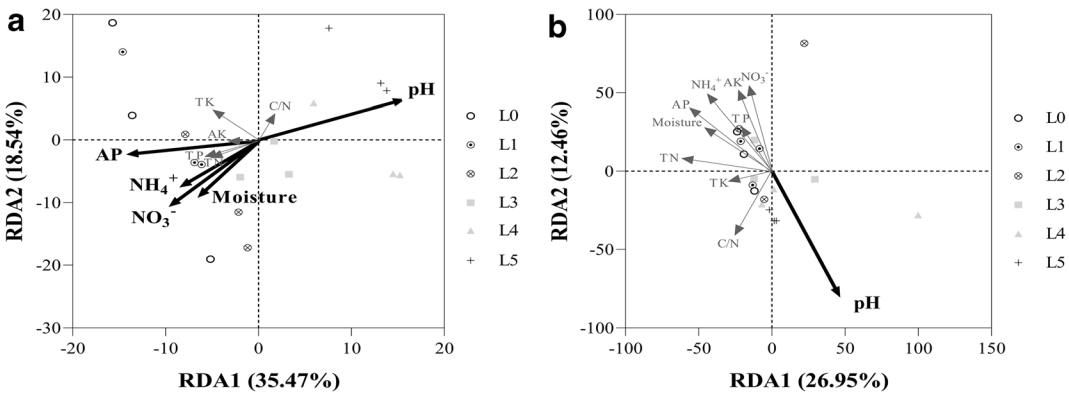

Fig. 6 Redundancy analysis of soil bacterial (**a**) and fungal (**b**) communities. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively

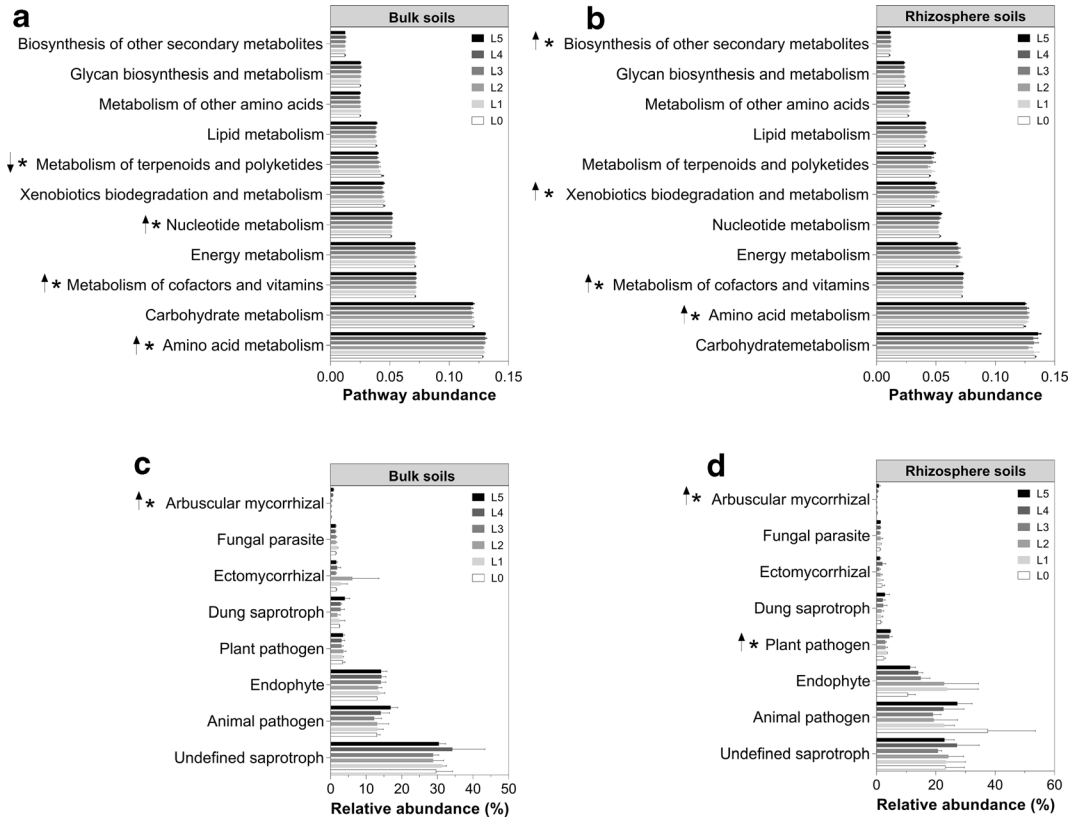

Fig. 7 Predicted functional profiles of bacterial and fungal communities. **a** KEGG (level 2 pathway) functional profiles of bacterial community in the bulk soils; **b** KEGG (level 2 pathway) functional profiles of bacterial community in the rhizosphere soils; **c** Guild assignments of fungal community in the bulk soils; **d** Guild assignments of fungal community in the rhizosphere soils. L0, L1, L1, L2, L3, L4 and L5 indicate the lime amendment rate at 0%, 0.04%, 0.08%, 0.16%, 0.32% and 0.48% of total soil mass, respectively

rhizosphere soils than in the bulk soils (Fig. 7c and d; Table S8). Moreover, the guild abundance of AM fungi was increased with the high level of lime addition in the bulk and rhizosphere soils ($P < 0.05$) (Fig. 7c and d; Table S8).

Discussion

Short-term lime amendment changes soil properties

The application of lime to acid soils resulted in a remarkable change on soil properties (Šiaudinis et al. 2017; Mkhonza et al. 2020). In this study, soil pH was positively correlated with lime amendment (Fig. S3), indicating that liming is an effective way to increase soil pH. We observed that soybean plant growth and soil microbial activity were increased with lime amendment (Fig. 1). This finding is consistent with previous studies showed that increasing plant growth and microbial activity by lime application would also improve root growth (Sing et al. 1987; Stenberg et al. 2000; Grewal and Williams 2003). However, it should be noted that the significant negative effect of liming was found on the soil factors of NO_3^--N, NH_4^+-N and AP contents (Table 1, Fig. S2). The application of liming to acid soils can improved the availability of reactive form of N and P for plant uptake (Haynes 1982; Mkhonza et al. 2020). In addition, increase of soil pH have significant effects on the soil N transformation processes (Nugroho et al. 2007; Liu et al. 2010a). Evidence shows that the positive correlation between liming and soil nitrification as well as N_2O fluxes, and liming can promote the process of NH_3 emissions, which may be one of the reasons resulting from the decrease of NO_3^--N, NH_4^+-N (Kemmitt et al. 2006; Baggs et al. 2010; Xue et al. 2020). We also found that soil moisture decreased along with increase of lime addition, suggesting that the

application of lime have an effect on soil moisture. Previous study showed that liming can improve soil aggregate stability and increase the hydraulic conductivity (Valzano et al. 2001). We speculated that the decrease of soil moisture may be related to the improvement of soil physical properties, which will help plants get more water for their own growth. To sum up, our findings illustrated that short-term lime amendment changed soil chemical properties, such as soil pH, NO_3^--N, NH_4^+-N, AP and soil moisture, which would impact on soil microbial community compositions.

Effect of liming on soil microbial abundance and diversity

Our results showed that lime application altered soil bacterial and fungal abundance in the bulk soils (Fig. 2), which was consistent with results of Zhang et al. (2020a), who reported that soil bacterial abundance was gradually increased as the soil pH value raising from 3.5 to 7.5 after 30 days of pH regulation, while the fungal abundance significantly decreased with the increase of soil pH value. In addition, liming only significantly increase bacterial abundance in the rhizosphere soil of L2, suggesting that liming may have a relative less effect on soil bacterial abundance in the rhizosphere. The lime application did not change the α-diversity in all soils (Fig. 4a-d), a similar result was also reported by Narendrula-Kotha and Nkongolo (2017), who found that microbial diversity index such as Simpson index, Shannon Index and Species evenness had no significant differences between limed and unlimed sites in the Greater Sudbury Region in Northern Ontario of Canada. This finding is inconsistent with common knowledge of soil bacterial α-diversity is positively related with soil pH in the range between 4 and 8 (Griffiths et al. 2011), since the soil pH was increased by adding lime (Table 1). In a long-term liming experimental field, Rousk et al. (2010) reported that the α-diversity of bacterial community was positively related to soil pH values. We speculate that the different results presented in this study may be due to the short-term application of lime. However, we observed that bacterial and fungal α-diversity present a great difference between bulk and rhizosphere soils (Fig. 4). The reason for this discrepancy might due to soil fungi grow more slowly than bacteria and have stronger tolerance to adversity (Rousk and Bååth 2007; De Vries et al. 2018). This finding also suggested that soil bacterial communities were more sensitive to changes in soil pH values and nutrient contents.

Effect of liming on soil microbial community structure

Although lime application did not significantly impact the α-diversity of bacterial and fungal communities in the bulk and rhizosphere soils (Fig. 4), there was a strong influence of lime addition on the β-diversity of the bacterial and fungal communities (Fig. 5). Correlation analysis corroborated the positive relationship between lime addition and CAP1 of bacterial and fungal communities (Fig. S3). This result was in line with the previous studies showed that the application of lime actually impacted soil microbial community structure (Mota et al. 2008; Xue et al. 2010; Ding et al. 2019). Several studies have demonstrated that environmental factors are important in shifting the composition of soil microbial communities (Erlandson et al. 2018; Li and Wu 2018; Zhang et al. 2020a). The finding of the significant relationship between pH and the bacterial and fungal community composition (Fig. 6; Table S5), suggesting that soil pH was the dominant soil properties in shifting the bacterial and fungal community structure (Lauber et al. 2009; Zhalnina et al. 2014). In addition, soil AP, NO_3^--N, NH_4^+-N and soil moisture also played an important role in the variation of soil bacterial community (Fig. 6a, b; Table S5), and all of these soil properties were significantly correlated with lime addition (Fig. 6, Fig. S2). These findings illustrated that soil microbial community structures were directly influenced by soil properties changed by lime addition.

Effect of liming on the functional profiles of soil microbial community

Lime addition significantly changed potential functional profiles of soil bacterial communities (Fig. 7a, b, Table S6), which is supported with finding of Pang et al. (2019), who observed that lime application improved soil bacterial functions in sugarcane fields. In addition, the metabolism pathways that can supply a lot of energy and nutrients to soil microorganisms for the life activities and growth such as methane metabolism of energy metabolism, citrate cycle (TCA cycle) and starch and sucrose metabolism of carbohydrate metabolism were significantly increased with lime addition in the rhizosphere soils, this may be the reason why the absolute abundance of microorganisms in the

rhizosphere soils was significantly higher than that in the bulk soils ($P < 0.001$) (Fig. 2). However, the KOs assigned to biosynthesis of siderophore group nonribosomal peptides, biosynthesis of ansamycins, biosynthesis of 12-, 14-, and 16-membered macrolides, sesquiterpenoid and triterpenoid biosynthesis as well as biosynthesis of vancomycin group antibiotics, which are related to terpenoids and polyketides metabolisms, were significantly decreased in the bulk soils ($P < 0.05$) (Table S7). Terpenoids and polyketides are the most purified antimicrobial secondary metabolites that are toxic to microbes, this provided evidence for the higher α-diversity of the bacterial community in the bulk soils (Mousa and Raizada 2013).

A surprising discovery in this study is that the guild abundance of AM fungi significantly increased with lime addition across all soil samples, suggesting that liming can actually help the growth of AM in a pH gradient ranging from 5.10 to 7.49 (Fig. 7c, d, Table S8). AM fungi are one of the most ecologically important fungal group including in the phylum of Glomeromycota, which can promote the plant growth in acid soils through enhancing the acquisition of nutrients such as nitrogen, phosphorus, sulfur, calcium and magnesium in plants (Clark and Zeto 2000; van der Heijden et al. 2015; Marro et al. 2020). By means of a designed microcosm unit, Hodge et al. (2001) illustrated that AM can strengthen decomposition and increase plant nitrogen capture from organic material in the soil. Moreover, AM fungi were able to interact with bacteria for stimulating plant growth through a series of mechanisms including enhancement of soil nitrogen and phosphorus availability (Artursson et al. 2006). This further supports our findings that inorganic nitrogen and phosphorus were decreased in the bulk soils and plant growth was significantly improved under the lime addition (Fig. 1a-c; Table 1).

Conclusions

Together, using a pot experiment, this study demonstrated that short-term lime application changed soil physiochemical properties, especially of soil pH, AP, available nitrogen nutrients (NO_3^--N, NH_4^+-N) and soil moisture. Soybean plant growth and soil microbial activity were improved by lime amendment, which also increased soil bacterial abundance but decreased fungal abundance in the bulk soils. However, no significant effects were observed on the changes of microbial abundance in the rhizosphere soils and soil microbial α-diversity across all samples. In contrast, short-term lime addition significantly changed soil microbial compositions and structures in both bulk and rhizosphere soils, and soil pH was the dominant soil properties in shifting bulk soil bacterial and fungal community structure. Furthermore, the several potential functions of soil bacterial and fungal communities were also influenced with lime application, especially amino acid, cofactors and vitamins metabolisms of the bacterial communities and arbuscular mycorrhizal of the fungal communities. Our findings have important implications for the understanding of the improvement of acidic soils by short-term liming.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-021-04913-0.

Acknowledgements This study was financially supported by the National Key Research and Development Program of China (No. 2017YFG200604) and National Natural Science Foundation of China (41977202).

Author contribution Guanghua Wang, Junjie Liu and Qin Yao designed this study. Sen Li performed the experiments, analyzed the data and wrote the manuscript. Zhenhua Yu, Yansheng Li, Jian Jin, and Xiaobing Liu participated in the experiments.Data availabilityThe raw sequences were deposited in the NCBI Sequence Read Archive under accession number PRJNA598057 and PRJNA598095 for the bulk and rhizosphere soil samples including bacterial and fungal communities, respectively.

Declarations

Conflicts of interest/competing interests The authors declare no conflict of interest exits.

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